



IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

PLANT PHYSIOLOGY

EDITORS

CHARLES A. SHULL

CHARLES B. LIPMAN

BURTON E. LIVINGSTON

CARLETON R. BALL

FRANCIS E. LLOYD

VOLUME 4

1929

WITH FIVE PLATES AND NINETY-SIX FIGURES



SCIENCE PRESS PRINTING CO.
LANCASTER, PA.

TABLE OF CONTENTS

NUMBER 1. JANUARY		PAGE
Studies in experimental cytology	CHARLES F. HOTTES	1
The influence of boron on the chemical composition and growth of the tomato plant	EARL S. JOHNSTON and W. H. DORE	31
Relation between top and root size in herbaceous plants.	JOHN W. CRIST and G. J. STOUT	63
The mechanism of the water tight door of the <i>Utricularia</i> trap.	FRANCIS E. LLOYD	87
Some critical comments on the methods in the expression of leaf saps.	BERNARD S. MEYER	103
Chemical relationship between scion and stock in <i>Citrus</i> .	A. R. C. HAYS and F. F. HALMA	113
Storage of truck crops: The girasole, <i>Helianthus tuberosus</i> .	HAMILTON P. TRAUB, CLIFFORD J. THOR, J. J. WILLAMAN and R. OLIVER	123
Seasonal variations of the chloroplast pigments in several plants on the Mall at Washington, D. C.	F. M. SCHERTZ	135
Effects of storage on alcoholic extracts of plant tissues. Amino acid changes	JAMES E. WEBSTER	141
X-ray photography of mineral accumulations in plants.	C. L. CRUTCHFIELD	145
JULIUS VON SACHS	R. B. HARVEY	155
An invading potato sprout	P. D. STRAUSBAUGH	157
SCHLOESING's experiments on the relation of transpiration to the translocation of minerals	W. E. LOOMIS	158
Notes		161
NUMBER 2. APRIL		
A study of the relations between chloroplast pigments and dry weights of tops in dent corn	H. B. SPRAGUE and J. W. SHIVE	165
The toxic substance produced by the eye-spot fungus of sugar cane, <i>Helminthosporium sacchari</i> Butler	A. HERTON LEE	193
The absorption of phosphate from soil and solution cultures.	L. J. H. TEAKLE	213
The extraction of nitrogenous materials from pear tissues.	F. B. LINCOLN and A. S. MULAY	233
Hemicellulose as a storage carbohydrate in woody plants with special reference to the apple	A. E. MURNEEK	251

Identification of certain species of <i>Citrus</i> by colorimetric tests.		PAGE
	F. F. HALMA and A. R. C. HAAS	265
✓ The effect of potassium, nitrogen and phosphorus fertilizing upon the chloroplast pigments, upon the mineral content of the leaves, and upon production in crop plants	F. M. SCHERTZ	269
The effect of temperature on flowers	F. M. ANDREWS	281
WILHELM PFEFFER	FRANK M. ANDREWS	285
✓ A simple method for nitrate nitrogen determination in wheat plants.		
	H. F. HOLTZ and CARL LARSON	288
Notes		293

NUMBER 3. JULY

On some limiting factors in the use of saturated petroleum oils as insecticides	HUGH KNIGHT, JOSEPH C. CHAMBERLIN and CHARLES D. SAMUELS	299
Opening of stomata in different ranges of wave lengths of light.		
	J. D. SAYRE	323
Mechanism of cell wall formation	O. L. SPONSLER	329
The pure pigments, carotin and xanthophyll, and the Tswett adsorption method	F. M. SCHERTZ	337
Growth studies on fruits. Respiration of tomato fruits.		
	FELIX G. GUSTAFSON	349
Some observations on the microchemical demonstration of phloridzin.		
	E. M. HARVEY	357
The effect of pH value and hydrogen peroxide concentration on fruit oxidase activity	W. V. CRUESS and W. Y. FONG	363
Testing the sugar content of beets for genetical purposes.		
	ERNEST REED	367
Effect of acid and alkaline hydrolysis on the estimation of hemicelluloses and associated groups in young apple wood.	FISK GERHARDT	373
Influence of shape on the chemical composition of potato tubers.		
	J. J. WILLAMAN and ALICE M. CHILD	385
✓ The water content of wheat at flowering time.		
	ANNIE M. HURD-KARRER and J. W. TAYLOR	393
Notes		399

NUMBER 4. OCTOBER

Composition and growth initiation of dormant Bartlett pear shoots as influenced by temperature	F. E. GARDNER	405
Relation between the development of root system and shoot under long- and short-day illumination.	J. E. WEAVER and W. J. HIMMEL	435
Some seasonal changes in the tracheal sap of pear and apricot trees.		
	F. G. ANDERSEN	459

	PAGE
Physical measurements of the winter wheat plant at various stages in its development	(GEORGE JANSSEN) 477
Some effects of calcium deficiency on <i>Pisum sativum</i> ..	DOROTHY DAY 493
The insoluble tyrosinase of the velvet bean seed coat.	EMERSON R. MILLER 507
The determination of nitrate in green tomato and lettuce tissues.	E. M. EMMERT 519
Moisture fluctuations in extracted plant solutions and in leaf tissue.	BASIL E. GILBERT and WALDO L. ADAMS 529
The effect of pH value on the inactivation temperature of fruit oxidase.	W. Y. FONG and W. V. CRUESS 537
JOANNES BAPTISTA VAN HELMONT	R. B. HARVEY 543
Notes	547

ERRATA

First cover page, vol. 4, no. 1, in first title, for on, read in.
 Page 136, first footnote to table, for *Gingko*, read *Ginkgo*.
 Page 152, legend to figure, for FeSO_4 , read K_2SO_4 .
 Page 157, line 13, for 1868, read 1865.
 Page 160, citation 4, for sans, read sous.
 Page 277, line 11 from bottom, for produces, read produce.
 Page 278, line 13, for considerable, read considerably.
 Page 279, citations 16 and 17, for chemiques, read chimiques.
 Page 287, last line, for build, read built.
 Page 302, footnote 1, for SAYBOLT, read SAYBOLDT.
 Page 303, title of table II, for transportation, read transpiration.
 Page 315, last column of table IV, sixth item, dele one word end.
 Page 316, line 10 from bottom, for droppd, read dropped.
 Page 403, line 12 from bottom, for \$9.00, read \$6.50.

PLANT PHYSIOLOGY

JANUARY, 1929

STUDIES IN EXPERIMENTAL CYTOLOGY

CHARLES F. HOTTES

(WITH FOUR FIGURES AND ONE PLATE)

I. Introduction

Cytologists have been too long content with the descriptive phase of their science. Cells of widely different character, and, consequently, in most diverse states or phases of physiologic activity, and prepared for study by the application of sundry methods, have served them for morphologic study and physiologic interpretation. This is responsible, in no small measure, (1) for the widely different results occasionally reported by different investigators working with supposedly identical material, and, (2) for the conflicting views now held with reference to the nature and the mechanism of the spindle, the behavior and function of the nucleoli, etc.—cell structures that have been the object of study since the beginning of the science.

The development of experimental cytology in recent years bids fair to lead us along lines of research affecting the intimate relation between the function and the structure of the cell organs, and through this relation to the discovery of the principles underlying cell activity. It has become quite generally recognized that the most noteworthy achievement in the progress of cell-study concerns, as one of the pioneers in experimental cytology says, “*die Methode der Forschung. Lange Zeit über eine rein deskriptive Wissenschaft, versucht die Zellenlehre in der Neuzeit den exakten Wissenschaften, Physik und Chemie, nachzueifern, die Erscheinungen nicht nur zu beschreiben, sondern ursächlich zu begreifen, einerseits auf dem Wege des Experiments, anderseits durch die Anwendung exakter Messmethoden*” (13, p. 2).

In following this newer line of cell-study one should never lose sight of the fact that during its period of activity the cell is subjected to a continuous series of chemical and physical processes induced by internal, and modi-

fied by external conditions or states. In the differentiated cell these follow a definitely fixed cycle in accordance with its specific functional activity; in the undifferentiated cell they pass through a regular progression of inter-correlated steps or phases as it advances to specific functional differentiation. Concomitant with the more or less fixed physiologic state of the differentiated cell, and the successive states of a meristematic cell in its progress toward physiologic fixity, may be noted a considerable morphologic detail. In the meristematic cell progressing toward specific functional differentiation the structure is constantly changing, and becomes fixed only when the cell assumes the permanent function demanded of it as a unit of the organism. This unit, in a given physiologic phase and under conditions such as usually prevail, possesses a specific structure and function which we call normal. The normal, in so far as it interests the cytologist, is the expression in cell structure of the harmonic interaction of the several cell organs. This interaction in the differentiated cell differs from that in the differentiating cell, and the cytologist is confronted by intergrading structures that are difficult or impossible correctly to interpret from a study of cells in a single physiologic phase.

The structure and function of an egg-cell is not that of a ganglion-cell, and the cell from the formative region of the root differs from one in the region of elongation. The degree and manner of reaction of these cells to chemical and physical agents, in so far as it can be followed in discernible structure and manifest function, is determined by the physiologic state or phase of the cell at the time of stimulation or subjection. But those conditions, chemical and physical, internal and external, which enable the cell, in consequence of the harmonic interaction of its organs, to perform its normal functions, that is, maintain the optimum of *cell* activity, are by no means identical with the conditions at which the *several cell-organs* in turn find their optima. The comparatively wide difference in the position of the cardinal points of activity—minimum, optimum, maximum—of the several cell-organs may be readily demonstrated by appropriate experiments. In the data later submitted and in the discussion that follows, it is apparent that the cytologist may, by carefully directed and controlled experiments, so affect the different cell-organs as (1) to excite or accelerate the activity of one while depressing or retarding that of another; (2) to modify completely the normal structure and function of one or several; and (3) to suppress the activity and destroy the structure of one or several. This furnishes an admirable opportunity to study the interrelations and interdependence of the several cell-organs, and must prove helpful in our endeavor to solve the intricacies of cell-processes, and of understanding cellular-derangements (pathologic) resulting from lack of proper coordination.

Plant physiologists have long recognized that the manner of response of meristematic cells to stimuli is influenced or determined by the physiologic state or phase of the cell at the time of stimulation. SACHS (38, p. 220) was the first clearly to recognize this important fact, and expressed it as follows: "Das Organ ist heute nicht mehr dasselbe Ding wie gestern und wird morgen wieder ein Ding mit anderen Eigenschaften sein, selbst wenn man dies an der äusseren Form und der mikroskopischen Struktur nicht wahrnimmt. Wird nun das sich entwickelnde, wachsende Organ von äusseren Einwirkungen betroffen, welche die Art seines Wachstums verändern, so muss diese Reaction verschieden ausfallen, je nach dem die gleiche äussere Einwirkung das wachsende Organ gestern, heute, oder morgen trifft."

Recent studies in experimental cytology and those here reported, most clearly demonstrate that the above statement applies equally well to the cell organs, and that in the future, greater consideration will be given to the physiologic state of the cell—tissue or egg—at the time of its fixation, or of its subjection to the action of external agents. Most of the work in experimental cytology has been done on cells in vastly different physiologic states, and the effect upon them of now one and now another stimulus of limited range of intensity and duration of action noted. This procedure gives us a mass of isolated detail, interesting and valuable in itself, but difficult or impossible to correlate. What we most need are studies that will enable us to coordinate the mass of unharmonized material we now have. Progress along this line of cytologic study is necessarily difficult and slow. For the present, we may profitably apply ourselves chiefly to an accurate and systematic experimental study of the behavior, toward physical and chemical agents, of the several cell-organs in cells of different physiologic states.

II. Materials and methods

The root-tip of *Vicia faba* was chosen because of its easy culture, its vigor, the readiness with which it responds to stimuli, and the large size of its cells and nuclei. Furthermore, it has been extensively used in cytologic study, and the recent investigations by LUNDEGÅRDH (24, 25, 27) on living material, give opportunity to appraise the respective values of results obtained by widely different methods of preparation for study. In a number of instances, the root-tips of *Zea mays*, *Phaseolus multiflorus*, and *Cucurbita pepo* were used for comparison. In the discussion that follows, all references, unless otherwise stated, pertain to the cells of the root-tips of *Vicia faba*.

GERMINATION

The seeds of *Vicia faba*, with the seed-coats previously broken to insure a more uniform swelling, were placed in tap water at 18 to 20° C. for 24

hours, the water being changed once. The swollen seeds were then placed in close proximity in a vertical position, with the micropyle lowermost, in a flat containing loosely packed, well-leached, moist sawdust. They were lightly covered with the same material, over which there was then spread a thin layer of dry sawdust to prevent excessive evaporation. A bed so prepared required no further watering and insured a rapid and normal germination. In from 50 to 60 hours at a temperature of 18 to 21° C., when the roots were from 3 to 4 cm. long, the seedlings were transferred on small-meshed paraffined wire netting to a jar of tap water for the purpose of accommodating the roots to an aqueous medium. The cotyledons resting on the wire netting immediately above the water of the jar, retained all necessary moisture for active translocation of materials by inverting over them a shallow second vessel of the same diameter as the jar. Diseased gelatinous tips, such as SACHS encountered when roots of *Vicia faba* were cultivated in well water for several hours, were never met with. For treatment only those roots that showed approximately the same rate of growth were used. The experiments were usually begun at eleven o'clock in the morning, since preliminary examination showed that the mitotic figures were then most abundant. At the beginning of each series of experiments, three of the root-tips of the seedlings about to be used were fixed as checks for comparison.

In an experimental study as here attempted the method of procedure is of primary importance, and the one outlined below was finally adopted and strictly followed. It is the direct outgrowth of unsuccessful attempts at correlation of results obtained by treating for a limited time, cells in different physiologic states, now with one, now with another agent.

THE PHYSIOLOGIC STATE

Cells of the same kind and origin but in different physiologic phases, were simultaneously subjected to the action of some physical or chemical condition or agent. The root-tip of *Vicia faba* with its clearly defined regions of cell multiplication, cell elongation, and cell differentiation, furnishes these conditions admirably.

THE INTENSITY OF THE ACTION

The physical and chemical conditions or agents were applied or supplied through a sufficiently wide range of intensity to obtain clear evidence of their specific effect on the cell organs, and to determine, when possible, the state of excitation or depression produced. By a sufficient extension of the intensity of the treatment, it was possible to determine the wide difference in reaction, adaptation, and finally, death-point of the several cell-organs;

and to observe differences, if any, in the manner or degree of response in the same organ in its different physiologic phases.

THE TIME OR DURATION OF ACTION

It is a well established fact in general physiology that the time, that is, the duration of action of an external agent is a factor of considerable importance. To know the effect of the agent applied for a brief period is not sufficient. We must learn something of the manner in which a cell-organ adjusts or adapts itself under certain prolonged conditions of action, and how these adjustments, in turn, affect, through correlation and interdependence of the organs, cell function and structure. Hence, in the experiments, the time or duration of action of the stimulus or agent was sufficiently prolonged (1) to allow a complete adaptation; or (2) to lead to complete exhaustion; or (3) to cause the destruction of a cell-organ.

To follow this cumulative effect it is necessary to use a large number of seedlings (100 to 200 for each series) and, while the conditions of the experiment are accurately maintained, to remove and fix at predetermined intervals, the root-tips of a given number.

RESTITUTION

Whenever from an experimental series roots were fixed for study, from 9 to 30 seedlings, similarly treated, were transferred to tap water at 18 to 20° C. for restitution. The successive stages in the process of restitution were followed by fixing three root-tips of the transferred seedlings at intervals of from 10 minutes to 1 hour. This phase of the experimental work has proven most interesting and has been a great help in the elucidation of some of the complex changes induced by the previous treatment.

NECROBIOSIS OR NECROSIS

When seedlings from a member of an experimental series transferred to water for restitution, showed, through death, that the damage done by the treatment was irreparable, *all* roots of that member in the series were excluded from further consideration. No attempt was made to follow the necrobiotic or necrotic (45, p. 162, 407, 412) changes in the severely injured cell, and such phenomena are not here considered. Our literature is by no means free from descriptions of such "submortem" (11, p. 160) structures.

SECTIONS OF LIVING ROOT-TIPS

Early in the development of our science FLEMMING, BÜTSCHLI, and others, recognized that the appearance of the several cell-structures, but more particularly that of the cytoplasm, may be due to the action of the

fixative or to the gradual changes occurring in the cell as it passes through successive stages from life to death—neurobiosis.

Later, with the idea of protoplasm as a colloid rapidly developing, the interesting and valuable experiments of HARDY (11) and FISCHER (6) firmly established this view, and, by the production, artificially, of structures appearing like those generally described by cytologists, placed in question much of the detail of cell-morphology and physiology.

Following HARDY and FISCHER we find a growing tendency to minimize the cumulative results obtained by cytologists using the fixation technique. By resorting to ingenious and skilful manipulation of materials bearing no direct relation to protoplasm, LEDUC (23, p. 121) and others have produced and explained (?) the mitotic process through the phenomena of osmosis, diffusion, coagulation, etc. LUNDEGÅRDH, paying little or no attention to these physio-chemical aspects, believes that the real solution of cell-structure must rest with the study of the living cell. By following this method only can the cytologist overcome and correct the errors for which his faulty technique is largely responsible.

In entering upon an extensive study of the influence of physiologic phase, and of physical and chemical agents on cell-structure, it becomes imperative that a technique above reproach should be employed.

From the criticisms made regarding the shortcomings of the fixation method, and the general recognition of the justification of such criticisms by cytologists, there seemed but one alternative, namely, the use of the direct method of observation in living material, or, at any rate, the use of living material as a check or arbiter in the interpretation of structural detail. Accordingly a serious and extensive study of living sections from both normal and treated root-tips was undertaken. The results from such a study were extremely disappointing, but it was thought that perseverance and training in the use of the new method would finally overcome the difficulties and lead to success. This optimism proved ill-founded, and after repeated trials and comparisons, the study from living material was abandoned. This, to some, will seem like heresy, and disregards the dictum of LUNDEGÅRDH (24, p. 237) "Und doch sind wohl keine wahren Fortschritte möglich, ohne dass man auf dem zuverlässigen Grund des direkt beobachteten baut."

The reasons that prompted the abandonment of the method of direct observation will be fully discussed under a separate heading. A few quotations from LUNDEGÅRDH's contributions will give, through admissions, some of the more apparent reasons, and will, it is hoped, hold prejudice in abeyance. "Über die allerfrühesten Stadien lässt sich am lebenden material keine bestimmte Auffassung bilden." (24, p. 275.) "Wenn auch—wie

gesagt—am lebenden Material sich eine ziemlich lückenlose Auffassung der Morphogenese des Karyotins während der Kernteilung gewinnen lässt, so ist doch die erzielte Erkenntnis nicht vollständig, und tatsächlich lässt einen das lebende material im Stich bei einer Anzahl wichtiger Vorgänge und Erscheinungen, wie z. B. der Metakinesis und den Anfangsstadien der Längsspaltung in den Spiremfäden." (24, p. 237.) "Die Art des Auseinandergehens der Chromosomen, der Teilung der Kernplatte, hat man nicht im Leben feststellen können." (24, p. 277.) "Sogar ganze Kerne können infolge der Lichtbrechungsverhältnisse unsichtbar sein und durch Hinzufügen von Mitteln, die die Wasserimbibitionsverhältnisse oder den Chemisch-physikalischen Zustand der Zelle verändern, sogleich zum Vorschein gebracht werden. In den Ruhekernen von *Vicia faba*, die in der Streckungszone liegen, sieht man häufig nichts ausser den Nukleolen. Nach Fixierung erweisen sie sich mit Karyosomen und Gerüst versehen, wobei Zahlenverhältnisse und Analogien mit den übrigen Kernen entschieden für die Präformation der ersteren sprechen." (27, p. 252).

FIXING AND STAINING

In order that the effects on the cell of the various agents employed throughout this series of experiments should be strictly comparable, a single fixing agent, HOF's (14, p. 67) modification of FLEMMING's chromic acid mixture, and but one stain, FLEMMING's safranin, gentian-violet, orange combination, were used. Of the FLEMMING mixture LUNDEGÅRDH (25, p. 214) says, "Ich habe in einigen Fällen den Vorgang unter dem Mikroskop verfolgen können, und grössere Umordnungen schienen wirklich dabei nicht einzutreten (ich spreche fortwährend von der FLEMMINGSCHEM Flüssigkeit)."

To aid in interpretation, and to check the method used in fixing and staining, the following were, in special cases, used: O. von ROTH's, GILSON's, and ZENKER's fixing agents, and ZIMMERMANN's fuchsin and iodine green, HEIDENHAIN's iron haematoxylin, and methylgreen. Frequently sections of treated and normal roots, of roots fixed in HOF's mixture and some one of the fixatives above mentioned, were placed together on the same slide and stained by one or another method. This was done to determine the action of the fixative and stain on normal and treated cells, and, more particularly, in the study of nucleoli, etc., to guard against "Spiegelfärbung" (6, p. 197).

The material remained in the HOF fixing agent for 30 hours, and was then washed in running water for 5 hours. It was dehydrated in alcohol of increasing concentration, cleared in chloroform, and imbedded in paraffin of 52° C. melting point. The sections were cut in ribbons, as a rule 5 μ in thickness, and fixed to the slide with MAYER's albumen fixative.

III. The cells of the normal root-tip

The cells of the normal root-tip when subjected to the various external agents and conditions later described, frequently show marked changes in structure and behavior. That these changes may be more readily recognized, a brief account of the cells of the normal root-tip is here given.

ZIMMERMANN (48), LAVDOWSKY (22), ROSEN (36), HOF (14), HOTTES (16), and GARDNER (8), described the metabolic, interphasic, and mitotic cell phenomena of *Vicia faba*. Although these earlier studies have been extended and amended by the work of FRASER and SNELL (7), of LUNDEGÅRDH (11, 19, 24, 25, 26, 27, 28), of SHARP (41), of SAKAMURA (40) and others, there still remain a number of points in controversy both as to detail of structure and interpretation. I feel confident that the experimental method used in the parts to follow will contribute evidence useful in reaching tenable conclusions regarding some of these mootable cases.

The manner and degree of structural change and functional reaction which the normal cell when subjected to chemical and physical agents undergoes, depends upon the physiologic phase of the cell at the time of stimulation. Hence a characterization of the cells of the three regions of SACHS is of primary importance. The region at the very tip with nearly trimetric cells filled with non-vacuolate cytoplasm, and with nuclei commonly in active mitotic division, is the region of cell formation; following it, with cells distinctly elongated, cytoplasm characteristically vacuolate, and with nuclei less commonly in mitotic division, is the region of elongation; finally with the elongated cells of the region just described, undergoing through internal differentiation the specific changes that lead to their mature structure and permanent function, is the region of differentiation. Since the above division of the cells of the root-tip into three regions is based entirely on the physiologic state of the cells, as was the intention of SACHS, it is impossible to locate the limits of the respective regions by distance from the tip of the root or the number of internodes from the tip of the stem. Failure to recognize this has led cytologists and ecologists into grave error. The length of the first and second zones is established by the influence of the factors of the environment on growth in length. Under the most favorable growth conditions the region of differentiation may be several centimeters removed from the tip, while under unfavorable conditions it is pushed acropetal to within a millimeter.

The cells of the formative region are filled with a dense, flocculent cytoplasm, near the center of which lies a spherical nucleus somewhat smaller than that of the slightly older cells. This interphasic nucleus has a reticulum of small meshes, somewhat irregular in form and size. Over this reticulum smaller and larger particles of chromatin are distributed. The

larger particles of irregular form, undoubtedly are identified with the bodies that LUNDEGÅRDH describes as karyosomes. They are by no means constantly present, and as SHARP (41, p. 309) has observed, show no longitudinal splitting as LUNDEGÅRDH reported. Occasionally the slightly irregular reticulum above referred to, is due to the faintly distinguishable outlines of the telophasic chromosomes in the process of transformation, as SHARP maintains. The varied appearance of the interphasic nucleus in the formative zone as well as in others, LUNDEGÅRDH (25, p. 228), is merely the morphologic expression of the rapid changes it undergoes from one physiologic phase to the next. In the experimental section will be noted the intimate and direct relation between these phases and the reaction of the cell to chemical and physical stimulation. Contrary to the statement of ROSEN (36, p. 265), the nucleus has a very distinct nuclear membrane. One or two nucleoli of spherical form, and never larger than one-third the diameter of the nucleus, lie in the center of a clearly defined hof. This hof differs in size, occasionally attaining a diameter equal to one-half that of the nucleus. Although very generally present in prepared material, it has been called an artifact by most cytologists, due to the refractive powers of the spherical nucleolus or to its shrinkage through poor fixation. Notwithstanding, the hof is here described as an intrinsic part of the structure of the nucleus and the convincing evidence of its reality will be presented in the experimental section. Mitotic figures in all phases are abundant and will be described later.

In the region of elongation the cells have sufficiently differentiated to enable one clearly to distinguish between the three rudimentary tissue systems. The cells of the dermatogen soon lose their meristematic character and simply elongate. In the younger regions these cells are still filled with a rather dense cytoplasm which becomes vacuolate as you pass to the next older. The nucleoli, one or two in number, are always small and frequently have a relatively large vacuole. The hof is either very small or is absent. The cytoplasm of the enlarging cells of the periblem increases very slightly or not at all and becomes vacuolate. A short distance beyond the typical formative zone, the greater part of the cell is occupied by a single vacuole. The nuclei in the younger portions of this zone reach their maximum size and remain nearly spherical until flattened by the pressure of the enlarging vacuole. The nucleoli also are larger than in the formative zone, though the increase is not in the same ratio as that of the nucleus. A hof is present in the cells of the periblem bordering on the formative zone. In the slightly older cells it is not uncommon to find threads of the reticulum to cross the hof to the nucleolus. Later chromatin granules may be found deposited on these threads. NĚMEC (32, p. 316) and others have

described similar threads crossing the hof. A lobed or toothed nucleolus resulting from the intimate contact of the threads with the nucleolus as LUNDEGÅRDH (24, p. 256) describes, was not observed; nor was a penetration of the nucleolus by the threads as stated by GARDNER (8, p. 174).

The cytoplasm of the cells of the procambial strands in the transition zone of the region of elongation, is flocculent and markedly denser than that of the younger cells of the root-tip. The nuclei in the long and narrow cells are usually elliptical in form, of large size, and furnished with a very dense reticulum over which numerous chromatin bodies, chromocentra, of varying size and shape, are distributed. The nucleoli are large, frequently elongated or lobed, and have one or more large vacuoles. A well defined hof is usually present. Amitoses as described by LAVDOWSKY (22) are absent.

The metabolic nucleus, virtually absent from the zone of cell formation, becomes increasingly prevalent in the older regions of the zone of cell elongation and of certain tissues of the zone of cell differentiation. It is in these regions, if anywhere, that its typical form should be sought, and not in the cells of the root-cap, LUNDEGÅRDH (25, p. 228). The cells of the root-cap very early pass through a series of intermediate stages, necrobiosis, from normal functional activity to more restricted activity and death. So, too, when he states (p. 228) that typical metabolic nuclei may be found throughout the cells of root-tips imbedded for a longer period in plaster of Paris, he fails to recognize that they are typical only for the cells of roots so treated. In fact, as will be shown later, their structure differs vastly from the nuclei found in the normal root-tips.

The structure of the metabolic nucleus is correlated with the physiological functions that it performs and the nature of the tissue of which it is a part. In a measure, they differ from one another to the degree in which their functions differ. Even a cursory examination of a longitudinal section of a root-tip will disclose the difference in the metabolic nuclei in the different zones, or of epidermis and plerome. The nucleus of the epidermal cell with its rather delicate and uniform reticulum, few and small chromocentra, small nucleoli and hof, and often cyanophilic reaction, stands in marked contrast to that of the cell of the future tracheal vessel with its coarse and often irregular reticulum, numerous and large chromocentra, large nucleoli and hof, and erythrophilic reaction.

The chromocentra have been previously described as pseudonucleoli, accessory nucleoli, chromatin nucleoli, prochromosomes, chromocentra, karyosomes, etc. They have been made the object of a more extensive study both in the living and fixed cell, by LUNDEGÅRDH (25) who suggested the term karyosome. To the use of this term TISCHLER (44, p. 65) finds

objection, and uses the term chromocentra proposed by BACCARINI (2). The number and size of the chromocentra is undoubtedly influenced by the metabolic state of the cells of *Vicia*, as was observed by ROSENBERG (37) and HUIE (17, 18) in the cells of the tentacles of *Drosera*. The observations of ROSENBERG and others [TISCHLER (44) p. 65] that the number of chromocentra—prochromosomes—as in *Capsella* is the same as that of the chromosomes, is not true for *Vicia*. The reticulum of the metabolic nucleus in these regions of the root-tip shows no areas of denser nature such as SHARP (41, p. 310) described and assumed to represent the chromosomes of the previous telophase, but is like that of FRASER and SNELL (7) and of LUNDEGÅRDH (25, 28).

In the early prophase the reticulum of the interphasic or metabolic nucleus gives way to a single slender thread that is much convoluted and becomes more and more basophilic as its development proceeds. This transformation takes place in the interphasic nucleus of short rest period after the manner described by SHARP (41, p. 311). The anastomosing branches of the nuclear reticulum are gradually withdrawn while the area of the reticulum constituting the telophasic chromosome undergoes condensation. In the less favorable stages of the interphase, and, more particularly, in the metabolic nucleus where no areas that might be designated as the telophasic chromosomes are observable, the condensation takes place into what appears as a continuous thread and shows no distinct ends of the chromosomes. [TISCHLER (44), p. 113].

The thread formed by one or the other method is not of uniform consistency or composition. That it varies in consistency is universally recognized; that it varies in composition is one of the most debated of nuclear phenomena [WILSON (47), SHARP (42)], and most difficult of solution. Its solution, for obvious reasons, is impossible from studies *in vivo*, and stains and micro-chemical reactions seemingly have increased our difficulties. Experimental cell-physiology apparently is destined to furnish the key. In its earlier and slender stage the thread shows a distinct transverse banding as first observed by PFITZNER in 1881 and since described in numbers of cases in the spirem of both plant and animal nuclei. The existence of these basichromatic bodies, chromomeres, in regular alternation with oxychromatic fields in the thread, is challenged by a number of observers. TISCHLER (44, p. 312) once an ardent supporter of the chromomere theory, closes his discussion as follows: "VAN WISSELINGH (1899) sowie GRÉGOIRE (1906, 1907) und seine Schule, MARTINS MANO (1904), STOMPS (1910), LUNDEGÅRDH (1912c, 1913a), SHARP (1913, 1920b, 1921, S. 155) zeigten aber m. E. unwiderleglich, dass es sich dabei oft um eine Verallgemeinerung einzelner Zufalls-Strukturen handle." After such a strong statement it

would seem folly to reopen the question. But a critical examination of the evidence given by TISCHLER and others, by no means shakes the confidence in the observations made on the normal cells of *Vicia* supported and extended by the experimental results later to be discussed. Structures that have been observed *in vivo* (CHAMBERS); that reappear with distinctness and in constant alternate succession on the spirem before condensation in numbers of plants and animals; that may be separated by the pull of the micro-dissection needle (CHAMBERS); that occur and reoccur in characteristic sequence, form, and position through successive generations in the spirem of *Phrynotettix* [WENRICH (46)]; that support and are supported by theories of heredity, and confirmed by experimental studies in genetics, are by no means "Zufalls-Strukturen."

A splitting of the chromocentra and their transformation into paired threads from which the chromosomes are built up as LUNDEGÅRDH (25, p. 256) describes, was not observed. The chromocentra gradually fade away in the manner described by SHARP (41, p. 312) and the material is incorporated with that of the developing skein. The thread now undergoes a longitudinal splitting, though the manner in which this occurs is not clear. Median vacuoles appearing as narrow slits such as described by NOTHNAGEL (33, p. 453) were not observed. Vacuolization in the normal chromosome of the telophase as CHAMBERLAIN (3, p. 209) describes, is evident, and in some of the treated cells later to be described, extremely marked. Following the longitudinal fission the thread becomes shorter and thicker, and forms into a dense mass surrounding the nucleolus. In this stage all trace of a split is lost through temporary fusion. In preparations not too densely stained and with strong light, the nucleolus, somewhat reduced in size and frequently irregular in outline, may still be seen in the center of the mass. While in this intimate contact with the chromatin thread, it rapidly decreases in size and finally entirely disappears through solution. The rate of solution differs with the nature of the cell, and is greatly affected by the environment. The idea of ROSEN (36, p. 272) "vielleicht spielen auch hier Ernährungs- und Wachstumsbedingungen der Organe, welchen die Kerne angehörten, eine Rolle" is confirmed in the experimental part.

While the thread shortens and thickens as described, the fundamentals of the spindle appear in the form of delicate kinoplasmic structures usually on opposite sides of the nucleus. In these fundamentals, at first low and dome-shaped, later conical, the kinoplasmic fibers may frequently be seen to diverge from several points (multipolar diarch), and later from a single point-bipolar diarch [STRASBURGER (43), p. 118]. According to DEVISÉ (5) and ROBYNS (35) these pole caps are of intranuclear origin and are intimately connected with the change in size which the prophase nucleus

undergoes. With the shrinkage of the nucleus in the later spirem stage, a polar flattening takes place due to the exudation from the nucleus of part of its karyolymph. This karyolymph meeting with the cytoplasmic fluid forms, through gelation, a homogeneous mass, the polar caps. The presence of fibers in these caps is attributed by them and others, to bad fixation.

For the present I must adhere to the statement above made, and agree with ALLEN (1, p. 281) that the spindle is of both nuclear and cytoplasmic origin, and that the fibers are real and perform a definite function in the mitotic process. A fuller discussion is reserved until the experimental evidence has been presented. When the closely wound thread opens and flattens into the equatorial plate, the paired superimposed chromosomes lie in a single plane. Repeated counts showed 2 long and 10 short [SAKAMURA (40)].

The spindle is now completely formed and consists, contrary to DEVISÉ (5), of two sets of fibers, (1) those of the central spindle running from pole to pole, and (2) the mantle fibers running from the place of insertion on the chromosome to the poles. The insertion of the fibers is near the middle of the 2 long and near the end of the 10 short chromosomes.

Slight deviations from the normal position of the mitotic figure are not infrequent in the cells of the normal root. They are largely due to space relations. In the broad but relatively short cells of the periblem, the mitotic figures not infrequently are placed diagonally in the cell and the chromosomes of the equatorial crown may not lie in a plane. By the rotation of the phragmoplast in the process of wall formation, the cell wall is usually formed perpendicular to the long axis of the root. It is clear that in such cases the polar caps do not indicate the polarity of the cell as maintained by ROBYNS (35).

The anaphase and telophase have been very fully described by others. They shall receive further attention after certain features contributed by the experimental section have been described.

Part I

1. The effects of pressure on the cells of the root-tip

A. ROOT-TIPS IMBEDDED IN PLASTER OF PARIS BLOCKS

The seedlings were grown in a manner already described, and when the roots had reached a length of 2 cm., they were thrust into a rather stiff mixture of plaster of Paris and water, as described by PFEFFER (34, p. 239). The plaster of Paris block must not be too small or the pressure exerted by the growing root will cause it to burst. The seedlings, with roots so imbedded, were placed in shallow pans of water at 18° to 20° C., and fixed at intervals of 24 hours for a period of 30 days. It was found that it would

not do to free the root from the plaster of Paris block before fixing. When freed before killing the root elongates considerably, as will be described later, and thus disturbs the true relations of the cells and cell structures. To prevent this, the seedling, with the block somewhat pared down, was immersed in the fixing agent for several hours for initial fixing. The roots were then freed of the adhering plaster of Paris and placed in fresh fixative for the usual time.

The changes occurring in the cells of imbedded roots during the first two days are marked; after that the difference is one of degree only. For description the cells of a root-tip imbedded for five days has been chosen.

The cytoplasm in all of the cells is greatly reduced in quantity, and is of a delicate flocculent structure. Rather coarse fibers staining deeply with the gentian-violet, are frequently found irregularly distributed through it. Occasionally, structures of a similar nature form a number of concentric rings with a small dense body in the center (fig. 1, pl. I). The regions of meristematic cells have been reduced fully one-half, and, in many respects, the cells present an appearance not unlike that of the ininitiated roots, to be described later. The zone of elongation has correspondingly decreased, and the tracheal tissue has moved acropetal to within 2.5 mm. of the tip [PFEFFER (34), p. 351; NATHANSOHN (29), p. 680], and the fundament of the youngest lateral root, to within 5 or 6 mm. of the tip. In the large vacuoles of many of the prematurely aged cells lie heaps of bodies staining orange.

The nucleus is smaller than in the corresponding physiologic zone of the normal root. Its reticulum is very coarse, dense, and basiphilous; here and there the individual meshes seemed to be enlarged, but, closer examination shows that this apparent enlargement is due to a branch of the greatly modified hof. This branch can readily be followed through the reticulum to the nuclear membrane. Where it meets the nuclear membrane this is raised in the form of a blister (figs. 1 and 2, pl. I), and gives the nucleus an amoeboid appearance.

The greatly reduced nucleolus, $1/3$ to $1/15$ the diameter of the normal, and spherical, disk, or lens-shaped, lies against the wall of the irregular hof. It stains normally and vacuoles are generally absent.

The cells near the tip continue to divide mitotically for approximately four days. Owing to the continuance of the nuclear and cell division without the possibility of elongation, the cells become greatly reduced in size (fig. 3, pl. I), and differ from those of the greatly shortened zone of elongation in that the walls are not thrown into folds (*entspannt*). The difference in the size of the cells that did not divide, as seen in the figure, and those that did, is easily recognized; also the difference in the size of the nuclei [*cf.* HALLBAUER (10) p. 15].

In the normal root the cells of the periblem very soon lose the power of division and then simply increase in size. If this increase be prevented, as by imbedding in plaster of Paris, the cell walls continue to grow by intussusception and are, therefore, thrown into folds, or are buckled, or send tube-like projections into the intercellular spaces. PFEFFER (34, p. 311, 321) first called attention to this buckling under conditions similar to those described above.

Apparently all growth soon ceases and the cells more than 2.5 mm. from the tip pass prematurely into permanent tissue. Mitotic divisions cease in the very short meristematic zone of the tip but will start anew very soon after the root is freed from the plaster of Paris cast. In this connection it is interesting to note the very rapid growth of the tip if, by chance, the root under high turgor pressure [PFEFFER (34), p. 300] pushes off an all too thin layer of the plaster and thus frees its tip. The first and very rapid elongation is due to the straightening of the buckled cell walls. The first apparent cellular changes consist in a slight increase in the quantity of the cytoplasm and the disappearance of the violet threads from the same. Then the nucleus slightly enlarges and regains its regular spherical form. The reticulum remains coarse and narrow meshed for some time, and chromatin in the form of chromocentra collects on the reticulum. The hof and nucleoli appreciably enlarge, and the irregular processes that the hof had sent to the nuclear wall disappear.

After 10 hours the mitotic figures reappear in increasing numbers. They are, except for size, in every respect normal. The spindle, owing to the irregular arrangement of the cells, is very frequently placed at an angle to the longitudinal axis of the root. The walls, however, are normally formed by the rotation of the phragmoplast.

When in the process of imbedding, air bubbles lodge along the course of the root, the small chambers thus formed are readily filled with tissue as shown in figure 4, plate I. Here the outermost layers of cells are more or less disorganized, while the cells of the first intact row are distinctly elongated in a direction perpendicular to the long axis of the root. This elongation becomes less marked in each successive row, and ceases in the third to the fifth row inclusive, as shown in figure 4, plate I. This growth—plastic growth—is the same as that responsible for filling in the intercellular spaces. Nuclear and cell divisions never occur in connection with the filling in of small chambers.

B. THE GROWTH OF ROOTS IN PLASTER MOLDS

The object of this series of experiments was to determine the manner in which lateral chambers in a mold, of various sizes and at different levels from the tip, were filled in by growth.

The molds were made as follows: Large flat plates of plaster of Paris 1 cm. in thickness were cast and then cut into blocks 3 x 2 cm. Two such blocks were placed with their smooth faces in contact and securely wired. By means of a steel instrument of approximately the same diameter as the root, a passage 2 cm. deep was so bored that one-half appeared in each section of the mold. The mouth of the passage was then enlarged as shown at *a*, figs. 1 and 2. The two halves of the mold were then separated and corresponding lateral chambers cut in the respective halves (figs. 1 and 2).



FIGS. 1 and 2. Molds of plaster of Paris for study of root growth.

The halves of the mold were then brought together again, reinforced by a piece of wood the size of the mold, and the whole securely wired together. A root about 2.5 cm. long was placed in the passage provided, and the widened mouth filled with plaster of Paris. The plaster of Paris served to keep the root in a fixed position and prevented it from backing out when the tip met with resistance. The heavy cotyledons were held in place by a pin thrust through them and into one of the blocks of wood. The seedlings thus prepared were immersed in tap water nearly up to the cotyledons. By the use of molds of this character, the roots may be made to assume almost any conceivable form, fig. 3.

The extent and nature of the growth by which the chambers of the mold are filled, vary with the form of the chamber and the distance it is placed from the tip of the root. A root secured in a mold as illustrated in fig. 2, soon reaches the end of the passage and is effectively hindered from further growth in length. For a number of days, approximately five, nuclear and cell division continue and form cells which in size are very much below the normal for the zone. While this is going on, the lateral chambers of the mold are gradually being filled with tissue by a localized

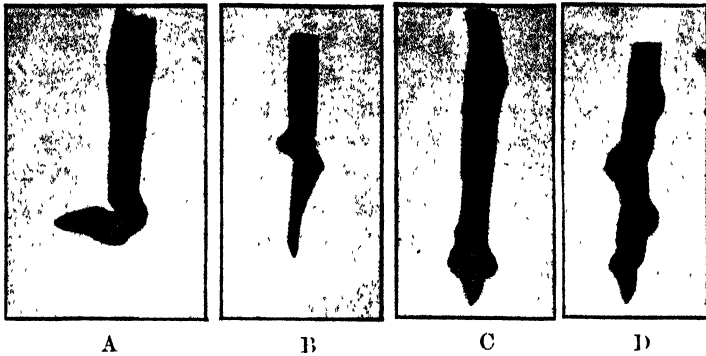


FIG. 3. Roots grown in molds similar to those of figs. 1 and 2. Outgrowths into lateral chambers shown in B, C, and D.

growth in thickness (fig. 3). This growth, by which ultimately an entire cavity is filled is distinctly of two kinds, the mode being determined by the distance of the cavity from the tip. Before entering upon a description of these two kinds of growth, it is essential that we have clearly in mind the nature of the cells, as described for root-tips imbedded in plaster of Paris blocks. The meristematic cells form a very short region of the tip, and the tracheal elements (permanent tissue) have moved acropetal to within 2 mm. of the tip. At this level, the periblem cells have lost the power of nuclear and cell division but still retain the power of growth as demonstrated by the folding or buckling of the walls. A lateral cavity situated in this region will, therefore, be filled by the elongation, laterally, of the cells of the periblem and a gliding of the cells past one another towards the area of least pressure (figs. 4 and 5, pl. I). The process may be compared to the filling of a mold with some plastic mass, and the term "plastic growth" may appropriately be applied [PFEFFER (34) p. 268].

In the region in which the tracheal elements have developed, a growth in thickness by nuclear and cell division very early takes place in the pericycle. This is demonstrated most readily by imbedding the tip of a root for only a few millimeters in plaster of Paris, thus preventing growth in length without checking growth in thickness. Such a root will show in a few days a marked increase in thickness on a level with the developed tracheal elements, but this gradually diminishes toward the older regions of the root. A microscopic examination will show this growth in thickness to be due to nuclear and cell division in the pericycle. In roots allowed to remain for some time thus fixed, a change in the rate and position of the thickening will take place. The reason for this becomes obvious when roots (fig. 3 D) grown in molds as illustrated in figure 2, are microscopically

examined. It is of interest to note that although the cavities are of equal size, the lateral growths of the root vary in size, decreasing in either direction from the third above the tip. A microscopical examination shows very clearly that the lateral growths from 1 to 2 mm. from the tip are produced by the enlargement laterally of the cells of the periblem, *i.e.*, by "plastic" and "gliding" growth. The chambers near the tip, if limited in size, are rapidly filled; those at a greater distance from the tip fill more slowly because of the different mode of growth. Here the filling in is due to nuclear and cell division taking place in the pericycle and pushing the tissue of the periblem before it. If the chamber is large enough, the tissue of the periblem is followed by the new tissue arising from this local and premature growth in thickness within the pericycle. The cell division in the pericycle will continue for some time after the chamber has been filled, but ceases when the already very narrow cells have been further reduced in size by cell division without the concomitant cell-stretching.

In contrast to the "plastic growth" earlier described, the growth just discussed may be designated as "correlation growth," [PFEFFER (34); HERING (12)] that is, a growth by nuclear and cell division that is directly related to and initiated through the interruption of the growth at the tip. At first sight, this correlation growth would seem to be nothing more than the normal growth in thickness. That this is not the case is readily seen by reference to figure 3 D. In this root the fourth lateral outgrowth, in a region in which the growth in thickness would normally be more active because the fundamentals are older and farther developed, is, nevertheless, considerably smaller than the second or third from the tip.

The cytoplasm, nucleus, nucleolus, and hof present the characteristics already described under A.

C. THE GROWTH OF ROOTS UNDER LATERAL PRESSURE

In the present section it is the intention to follow the method of growth, and, more especially, to determine the position of the mitotic spindle in roots of *Vicia faba* placed under strong lateral pressure.

KNY was the first experimentally to follow a modification of growth in plant tissue as determined by lateral pressure. "Sie geben für das Pflanzenreich den ersten experimentellen Nachweis, dass es möglich ist, die Orientirung der Kernfigur und damit die der Theilungswand dadurch zu bestimmen, das man durch Zug bezw. Druck dem vorhergehenden intensivsten Wachsthum eine bestimmte Richtung willkürlich aufnöthigt" [KNY (19), p. 390]. It must be here stated that KNY reached the above conclusion from a study of the position of the cell walls; the orientation of the

mitotic figure was not observed. NĚMEC (30, p. 214) repeated the experiments of KNY and paid particular attention to the position of the mitotic figure. He confirms KNY's results in so far as the orientation of the cell wall is concerned, and adds that the pressure experimentally applied, modifies the periplast (p. 242) and through it determines the orientation of the mitotic spindle, and, consequently, the cell wall. He says, "Ich wiederholte einige Versuche, die in dieser Beziehung von KNY ausgeführt wurden (mit Kartoffeln, Wurzeln von *Helianthus annuus*, *Pisum*, und *Vicia*) und fand, dass durch Zug oder Druck die bipolare hyaline Spindelanlage oder wenigstens die Achse der achromatischen Fäserchen orientirt wird. Die Richtungen dieser Achsen stimmen aber mit den Richtungen der geringsten, durch Zug oder Druck eines homogenen festen Körper inducirten Elasticität überein." [NĚMEC (31), p. 247]. From the above and also from his discussion on page 242, it is apparent that NĚMEC assumes that the orientation of the spindle in the cells of roots growing under lateral pressure is due to a modification of the form of the "periplast." The periplast with its fluid content is spherical when in complete equilibrium, and becomes elliptical through lateral pressure acting on the cytoplasm. From this one may infer that NĚMEC supposed the cytoplasm to be equipotential, that is, isotropic, and that the change in the form of the periplast is due to a change in the cytoplasm in consequence of which it fails to transmit pressure as does a liquid.

In a second contribution KNY again takes up the influence of pressure on cell division and, so far as pertains to his study of the root-tip of *Vicia faba*, still holds to his former view, though he modifies it as follows: "Es zeigt sich hier, wie auch an anderen Stellen des Wurzelquerschnittes, dass die Druckkräfte den durch Erblichkeit vorgezeichneten Zelltheilungsvorgängen bis zu einem gewissen Grade entgegenwirken, sie aber nicht vollständig unterdrücken" [KNY (20) p. 77].

The method used for placing the root under lateral pressure is a modification of the one used by KNY in his earlier work [HOTTES (16), p. 41]. Two pieces of plate glass 12.5 cm. square are held together by wooden strips in such a manner that while they meet along their lower edges there is a distance of about 3 mm. between the upper edges. This distance is maintained by the insertion of small cork plugs (fig. 4). The width of the wedge formed by the two plates could be readily varied by simply sliding the wooden strips up or down. Between the two wooden strips on one side was placed a rather strong nicked steel spring. This gives a constant pressure upon the growing tip.

The seedling was placed in position by carefully shoving the root between the converging plates until its tip met with a considerable resistance. The

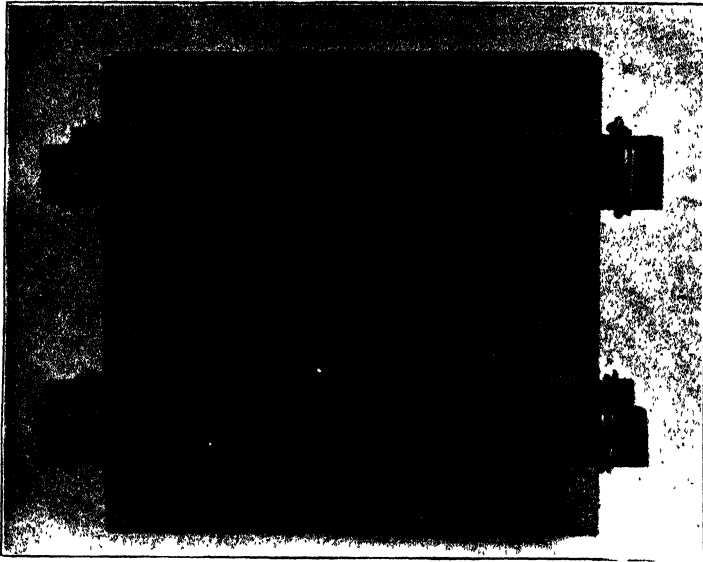


FIG. 4. Glass plate apparatus for producing lateral pressure upon growing roots.

whole was then placed in a dish of tap water reaching nearly to the cotyledons. The apparatus was slightly inclined until the root-tip had by growth wedged itself between the glass plates, when it was placed in a vertical position. Curvature of the tip was by this greatly reduced. When the root had firmly established itself, the wooden bars were moved upward sufficiently to slightly compress the spring. In the wider portion of the wedge, the root grew quite rapidly, but when it reached the portion where the plates were only 0.5 mm. apart, growth in length was greatly retarded and soon ceased. With this retardation or cessation of growth in length, a growth in thickness, parallel to the plates would very soon manifest itself. This latter growth reached its maximum about 3 mm. from the tip, and then decreased in both directions, though the more rapidly toward the tip. This zone of maximum growth agrees perfectly with the growth in thickness described under A and B as "correlation growth."

For reasons similar to those stated under A, it was found necessary to kill and partially fix the root-tips before freeing them from the glass wedge.

Cross-sections at different levels of root-tips grown as above described, agree with the results reported by KNY and NĚMEC in so far as size relations and number of cells are concerned. In several cases the difference in the number of cells parallel and perpendicular to the glass plates was considerably larger than that given by these investigators. Although the number of cells parallel and perpendicular to the glass plates of the wedge is essen-

tially that reported by KNY and NĚMEC, I am convinced that this difference, as also the orientation of the cell walls and mitotic figure, can be accounted for by the forms of growth discussed under A and B.

When the root growing into the ever-narrowing wedge finally reaches its limit of growth, the cells of the tip and of the periblem perpendicular to the glass plates show essentially the same characters as described under A. The cells of the tip when further advancement of the root-tip is mechanically prevented, continue to divide mitotically but fail to elongate in the normal axial direction. A high turgor pressure is developed in such cells, and, in consequence of it, the cell is deformed by sending processes into the intercellular spaces. On the free sides, that is, the sides opposite the pressure, elongation is more extensive and the cells more regular in size and shape. A cross-section of such root-tips frequently shows distinct evidence of a gliding growth. The cells in the diameter perpendicular to the glass plates are soon mechanically prevented from farther elongation; those in the diameter parallel to the glass plates are free to elongate in that direction. In the angles formed between these cell complexes, the lines of a gliding growth are very apparent (fig. 6, pl. I). By careful comparison of the cross-sections of a large number of roots, I find that the mitotic figures, so long as cell division in the tip and the periblem continue, are normally oriented. The difference in the number of the cells on the sides under pressure as compared with those not under pressure is not due to the orientation of the spindle through pressure. From a study of longitudinal sections of roots treated as above, it is very evident that the cause must be attributed, for the greater part, to plastic and gliding growth (fig. 7, pl. I). In addition to this, correlation growth brought on by the cessation of division in the cells that are mechanically prevented from enlarging and the acropetal movement of the permanent tissue plays an interesting and important part. Under A and B it was shown that cell division in both the tip and the pericycle, ceased when the cell was reduced in size by nuclear and cell divisions without the concomitant stretching.

In roots subjected to lateral pressure, a growth in thickness very soon starts. This shows the same peculiarity as that already described in sections A and B, being most rapid at a point a little back of the end of the tracheal elements. This growth, "correlation growth," is due to the multiplication of the cells of the pericycle, perpendicular to the long axis of the root, and is induced by the retardation or cessation of the growth of the tip. The normal growth in thickness, starting later, will eventually overtake that described as correlation growth. In both these modes of growth in thickness the mitotic spindles are normally oriented, that is, perpendicular to the long axis of the root. On the sides of the root not subjected to

pressure nuclear division and growth continue; while on the sides subjected to pressure the nuclear and cell divisions cease when the cells reach a minimum size.

The most interesting and conclusive evidence in support of the above is offered by the study of longitudinal sections of a root grown under pressure. If longitudinal sections perpendicular to the glass plates be made to the center of the root, and the root reimbedded and cut in a plane parallel to the glass plates, the cells in the pericycle will appear respectively as represented in figs. 8 and 9, pl. I. In 9, the nuclear and cell divisions have continued until a large number of rather small and closely packed cells have been produced. The mitotic figures, as represented, are normal in position, and at this time, because of the few cells of even average size, very few in number. Soon, all divisions in this plane will cease. Cell division and growth, however, will continue in a direction parallel to the glass plates.

The mitotic figures in every phase appear normal, and but for occasional exceptions due to irregularities of the cells on account of varying space-accommodation, are placed perpendicular and not parallel to the glass plates. In no case examined could there be found any indication of an effect on the early mitotic phases such as NĚMĚC describes (31 p. 246).

The cells of roots treated as described in sections A, B, and C show many points of similarity. By the methods of experimentation used, the roots in each case were mechanically prevented from growth in length. This inhibition affected both the internal structure of the cell, and the manner and rate of growth in thickness of the root.

The cytoplasm of the tip cells is rapidly reduced in quantity, though its structure is not materially changed. In the cells of the formative region, it has been reduced from one-half to two-thirds its normal extent, and peculiar heavy violet threads or concentric circles are quite common.

The cells of the periblem lose their cytoplasm far more rapidly than do the cells of the plerome or the initial cells of the tip. This loss of cytoplasm, however, does not affect the turgor of the cells as has been shown [PFEFFER (34)], and as will appear in a subsequent discussion. In the cells in which the turgor has been greatly increased large numbers of spherical bodies are usually found.

The nucleus of the formative region is somewhat smaller than that in normal cells of similar position and size. The reticulum is very dense, owing to its fine meshes and apparently abundant chromatin. The nucleolus is greatly reduced in size, varying from $1/3$ to $1/15$ the diameter of the nucleolus in similar cells of the normal root. The hof is absent or greatly reduced in size, and frequently sends an irregular canal to the

nuclear membrane. At the point where this canal meets the nuclear membrane the latter frequently shows a papilla-like protrusion.

Nuclear and cell divisions continue for some time in the formative region even when the cells are mechanically prevented from further growth (fig. 3, pl. I). The size of the nuclei in these newly formed cells is distinctly smaller than in the contiguous larger cells. This with the reduction in quantity of the cytoplasm already stated are of interest in connection with the "nucleus-plasma-relation" of HERTWIG (13, p. 4). The nuclear and cell divisions soon cease, and the metabolic nucleus remains apparently unchanged for a period of 30 days. Up to and including this period, the roots begin at once to elongate when freed from the mechanical hindrance. This elongation is due to the high turgor pressure in cells with buckled walls. Nuclear and cell divisions occur in from 5 to 24 hours. The division of the nucleus is always preceded by a slight increase in the cytoplasm. The mitotic figure is normal, but undersize.

All the mitotic figures both in the formative region of the tip and in the pericycle are normal, except for size. In no case was evidence found in support of the aggregate condition of the cytoplasm, which by transmitting pressure unlike liquids, exerts an orienting influence on the mitotic figure through a deformation of the periplast [NĚMEC (31), p. 226]. The orientation of the mature spindle is normal, and the difference in the number of cells, etc., parallel and perpendicular to the plane of pressure [cf. HOTTES (16) p. 43] is due to plastic and gliding and correlation growth [KÖHLER (21)].

In a root whose tip is prevented from further growth, the tracheal tissue moves acropetal to within 1 or 2 mm. of the tip, and a correlation growth in the pericycle very soon starts.

When the cells of the periblem have lost the power of nuclear division, they are still in a stage of active growth.

In roots subjected to lateral pressure between two glass plates, active cell growth in the periblem continues on the free sides. This is exactly comparable to the filling of the cavities in plaster of Paris molds as described under B.

If further growth of the tip of the root is mechanically prevented by the converging glass plates and cell division has ceased, the walls of the cells continue to elongate by intussusception and are thrown into folds, or buckle, as shown in figure 4, pl. I. This buckling of the membrane takes place while a very high turgor is maintained in the cells [PFEFFER (34), p. 296], and suggests further work on the relation between turgor and growth by wall-stretching [DAVENPORT (4), p. 73]. By this process of plastic growth the regular arrangement of the cells becomes disturbed to a greater or less

degree and a process of gliding growth ensues as at *a*, fig. 7, pl. I. This gliding growth, so manifest in longitudinal section, accounts for the following statements by KNY: "Die Rindenzellen greifen unregelmässig zwischen einander ein" (20, p. 77). This growth, however, is of relatively small interest as compared with that due to nuclear and cell division in the pericycle. Growth in thickness normally begins in the older and proceeds to the younger regions of the root. When growth in length is prevented, a growth in thickness in the younger regions lying from 2 to 5 mm. back of the tip begins. This produces a swelling, which falls off abruptly toward the tip and more gradually in the opposite direction. Such an abrupt and local growth in thickness was obtained in roots of *Vicia* many times and in different ways. In fact, any agent that destroys or inhibits growth at the tip, is likely to produce this local thickening through correlation growth. Figure 10, pl. I, represents a root of *Vicia* that was grown in moist air and dipped in cold water for an instant every day. SACHS (39, p. 801) produced similar roots by the same method. He further observed a similar thickening in a root of *Monstera*, which, while growing along horizontally, struck with its growing tip a rough wall. HOFMEISTER (15, p. 161) before SACHS, had observed a similar thickening when roots of *Zea mays* grew against the bottom of the flower pot. Both HOFMEISTER and SACHS at first attributed this thickening to a telescoping of the tender cells when the tip is mechanically stopped and growth in the regions behind it continues. When SACHS obtained similar results on roots in moist air, he gave up this idea without attempting an explanation.

From the above it is apparent that there exists a definite relation between the retardation or inhibition of the growth of the tip and this peculiar mode of growth in thickness. Further, by alternately recurring conditions, now favorable, now unfavorable to growth in length, a more or less moniliform root may be produced (fig. 10, pl. I). It is apparent, then, that this form of growth is due to stimulation, and PFEFFER's term, "correlation growth" may be applied to it.

Anatomical and cytological studies show conclusively that this mode of growth is due to premature nuclear and cell divisions in the pericycle induced through stimulation. The orientation of the spindle, and consequently the position of the cell wall, is not determined as KNY, NĚMEC, and GIESENHAGEN (9, p. 45) maintain. The tissue differentiation within the root-tip whose growth is mechanically or otherwise retarded or inhibited, determines through correlation the character of the subsequent growth, and, consequently, the orientation of the mitotic spindle. This premature growth in thickness in the region near the tip starts in roots imbedded in

plaster of Paris, or growing between glass plates, but soon ceases with the reduction in the size of the cells, and is easily overlooked. It can be readily detected if chambers are provided in the region of the root immediately behind the tip, as at the second and third lateral growths from the tips in figure 3 D. In roots subjected to lateral pressure, as described under C, few nuclear and cell divisions occur perpendicular to the plates because of the rapid decrease in the size of the cells. In such as do occur, the orientation of the mitotic spindle is normal, that is, the axis of the figure coincides with the radius of the root. On the sides not under pressure this growth in thickness continues and forms a considerable tissue. The manner of growth is not readily followed in cross-sections, as KNY and NĚMEC evidently studied, but in longitudinal sections prepared as described under C, and as represented by figures 8 and 9, pl. I, is easily apparent.

What has been said of the spindle in "correlation growth" holds equally well for the normal growth in thickness further removed from the tip. The statement of KNY, "Bei Zelltheilungen suchen sich die Scheidewände in der Richtung des Druckes und senkrecht zur Richtung des Zuges zu stellen" (20, p. 96), does not agree with the observations here recorded on *Vicia*.

Summary

In roots of *Vicia faba* whose tip is mechanically inhibited from further elongation, nuclear and cell division continue for approximately five days.

Nuclear and cell division cease when the cell reaches a minimum size.

The turgor in such cells may rise many fold above that of normal cells of similar age and position.

Nuclear and cell division in cells of minimum size may again recur when the inhibiting agent is removed, and the interval following the inhibition has not been unduly prolonged.

Turgor, perhaps, more than minimum size, is the inhibitor of nuclear division.

The inhibition of nuclear and cell division and of elongation promotes internal tissue differentiation.

The tracheal tissue may move acropetal to within 2 mm. of the tip.

The premature internal differentiation starts nuclear and cell division in the pericycle and leads to a growth in thickness induced through correlation.

The mitotic figure in every instance is normal in form and orientation.

In cross sections of roots under lateral pressure the number of cells parallel and perpendicular to the line of pressure is due to plastic and gliding and correlation growths.

The radially placed spindle and the resulting cell walls are identically formed and oriented in the earliest stages of correlation growth induced by the inhibition of longitudinal growth.

In the later phases nuclear and cell division cease on the sides under pressure and continue on the free sides.

This leads to a greater number of cells on the free sides and the orientation of the cell wall apparently in accordance with the views of KNY and NĚMEC.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF ILLINOIS.

LITERATURE CITED

1. ALLEN, C. E. The early stages of spindle-formation in the pollen-mother-cells of *Larix*. Ann. Bot. **17**: 281-312. 1903.
2. BACCARINI, J. Sulle cinesi vegetative del *Cynomorium coccineum* L. Nuovo giorn. bot. ital. n. ser. **15**: 189-204. 1908.
3. CHAMBERLAIN, CHARLES J. Comparative morphology of cytoplasm and chromatin. Bot. Gaz. **80**: 203-212. 1925.
4. DAVENPORT, C. B. The rôle of water in growth. Proc. Boston Soc. Nat. Hist. **28**: 73-84. 1897.
5. DEVISÉ, R. La figure achromatique et la plaque cellulaire dans les microsporocytes du "*Larix europaea*." La Cellule **32**: 249-303. 1922.
6. FISCHER, ALFRED. Fixirung, Färbung und Bau des Protoplasmas. Jena. 1899.
7. FRASER, HELEN C. I. and SNELL, J. The vegetative divisions in *Vicia faba*. Ann. Bot. **25**: 845-855. 1911.
8. GARDNER, BLANCHE. Observations on growth and cell division in the root of *Vicia faba*. Pub. Univ. Pennsylvania. n. ser. no. 6. **2**: 150-182. 1901.
9. GIESENHAGEN, K. Studien über die Zellteilung im Pflanzenreiche. Stuttgart. 1905.
10. HALLBAUER, W. Ueber den Einfluss allseitiger mechanischer Hemmung durch einen Gipsverband auf die Wachstumszone und die innere Differenzierung der Pflanzen. Diss. Leipzig. pp. 73. 1909.
11. HARDY, W. B. On the structure of cell protoplasm. Jour. Physiol. **24**: 158-210. 1899.

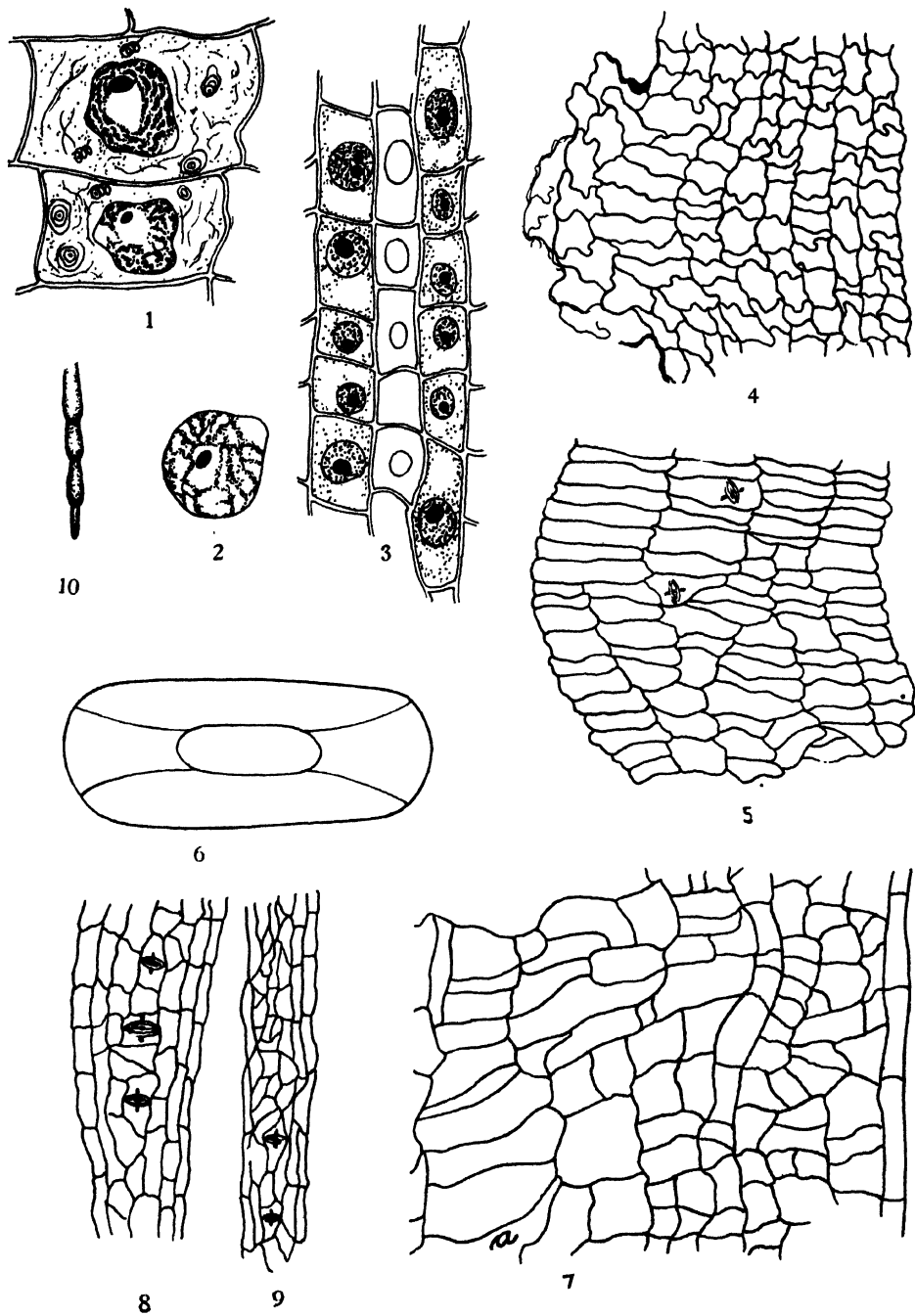
12. HERING, F. Ueber Wachstumsrelationen in Folge mechanischer Hemmung des Wachsens. Jahrb. wiss. Bot. **29**: 132–170. 1896.
13. HERTWIG, R. Über neue Probleme der Zellenlehre. Archiv. Zellforsch. **1**: 1–32. 1908.
14. HOF, A. C. Histologische Studien an Vegetationspunkten. Bot. Centralbl. **76**: 65–69. 1898.
15. HOFMEISTER, W. Handbuch der physiologischen Botanik. vol. 1. Leipzig. 1867.
16. HOTTES, C. F. Ueber den Einfluss von Druckwirkungen auf die Wurzel von *Vicia faba*. Diss. Bonn. 1901.
17. HUITE, L. H. Changes in the tentacle of *Drosera rotundifolia* produced by feeding with egg albumen. Ann. Bot. **10**: 625–626. 1896.
18. ———. Further studies of cytological changes produced in *Drosera*. Quar. Jour. Microscop. Sci. n. ser. **42**: 203–222. 1899.
19. KNY, L. Ueber den Einfluss von Zug und Druck auf die Richtung der Scheidewände in sich theilenden Pflanzenzellen. Ber. deutsch. bot. Ges. **14**: 378–391. 1896.
20. ———. Ueber den Einfluss von Zug und Druck auf die Pflanzenzellen. Zweite Mitt. Jahrb. wiss. Bot. **37**: 55–98. 1902.
21. KÖHLER, R. Ueber die plastischen und anatomischen Veränderungen bei Keimwurzeln und Luftwurzeln hervorgerufen durch partielle mechanische Hemmungen. Diss. Leipzig. 1902.
22. LAVDOWSKY, M. Von der Entstehung der chromatischen und achromatischen Substanzen in den tierischen und pflanzlichen Zellen. Anatom. Hefte **4**: 355–446. 1894.
23. LEDUC, S. Théorie physico-chimique de la vie. La biologie synthétique. 1912.
24. LUNDEGÅRDH, H. Die Kernteilung bei höheren Organismen nach Untersuchungen an lebendem Material. Jahrb. wiss. Bot. **51**: 236–282. 1912.
25. ———. Das Caryotin im Ruhekern und sein Verhalten bei der Bildung und Auflösung der Chromosomen. Archiv Zellforsch. **9**: 205–330. 1912–1913.
26. ———. Chromosomen, Nukleolen und die Veränderungen im Protoplasma bei der Karyokinese. Beitr. Biol. Pflanzen. **11**: 373–542. 1912.
27. ———. Fixierung, Färbung und Nomenklatur der Kernstrukturen. Archiv mikroskop. Anat. **80**: 223–273. 1912.
28. ———. Über Kernteilung in den Wurzelspitzen von *Allium cepa* und *Vicia faba*. Svensk Bot. Tidskr. **4**: 174–196. 1910.

29. NATHANSOHN, A. Beiträge zur Kenntniss des Wachstums der trachealen Elemente. Jahrb. wiss. Bot. **32**: 671–686. 1898.
30. NÉMEC, B. Ueber Kern- und Zelltheilung bei *Solanum tuberosum*. Flora **86**: 214–227. 1899.
31. ———. Zur Physiologie der Kern- und Zelltheilung. Bot. Centralbl. **77**: 241–251. 1899.
32. ———. Ueber die karyokinetische Kerntheilung in der Wurzelspitze von *Allium cepa*. Jahrb. wiss. Bot. **33**: 313–336. 1899.
33. NOTHNAGEL, MILDRED. Reduction divisions in the pollen mother cells of *Allium tricoccum*. Bot. Gaz. **61**: 453–476. 1916.
34. PFEFFER, W. Druck und Arbeitsleistung durch wachsende Pflanze. Abhandl. K. Sächs. Gesell. Wiss. **20**: 235–474. 1893.
35. ROBYNS, W. Le fuseau de caryocinèse et le fuseau de cytocinèse dans les divisions somatiques des Phanérogams. La Cellule **34**: 367–454. 1924.
36. ROSEN, F. Beiträge zur Kenntniss der Pflanzenzellen. Beitr. Biol. Pflanzen. **7**: 225–313. 1896.
37. ROSENBERG, O. Physiologisch-cytologische Untersuchungen über *Drosera rotundifolia*. Diss. Bonn. 1899.
38. SACHS, J. VON. Ueber Wachstumsperioden und Bildungsreize. Flora **77**: 217–253. 1893.
39. ———. Gesammelte Abhandlungen über Pflanzenphysiologie. Leipzig. 1893.
40. SAKAMURA, T. Experimentelle Studien über die Zell und Kernteilung mit besonderer Rücksicht auf Form, Grösse, und Zahl der Chromosomen. Jour. Coll. Sci. Imp. Univ. Tokyo **39**: Art. 11. 1–221. 1920.
41. SHARP, L. W. Somatic chromosomes in *Vicia*. La Cellule **29**: 297–331. 1913.
42. ———. An introduction to cytology. 2nd ed. McGraw-Hill. 1926.
43. STRASBURGER, E. Ueber Reductionstheilung, Spindelbildung, Centrosomen und Cilienbildner im Pflanzenreich. Histologische Beiträge **6**: 1–224. 1900.
44. TISCHLER, G. Allgemeine Pflanzenkaryologie. pp. 899. Berlin. 1921.
45. VERWORN, MAX. Allgemeine Physiologie. pp. 816. Jena. 1922.
46. WENRICH, D. H. The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zoology, Harvard Coll. **60**: 55–136. 1916.

47. WILSON, E. B. The cell in development and heredity. 3rd ed. Macmillan. 1925.
48. ZIMMERMAN, A. Über das Verhalten der Nukleolen während der Karyokinese. Beiträge Morph. Physiol. Pflanzenzelle **2**: 1–35. 1893.

EXPLANATION OF PLATE

- FIG. 1. Cells from imbedded root, showing characteristic cytoplasmic features. Note concentric ring structures surrounding a small dense body.
- FIG. 2. Nucleus with amoeboid appearance, the nuclear membrane raised in the form of a blister where a branch of the modified hof meets the nuclear membrane.
- FIG. 3. Cells reduced in size by continued nuclear and cell division, without elongation. Note the smaller nuclei in the recently formed smaller cells.
- FIG. 4. Cells with walls thrown into folds by pressure. The buckling of the walls occurs along with high turgor in the cells.
- FIG. 5. Growth behavior of cells in "plastic" growth. Periblem cells elongate laterally, gliding past one another towards the area of least pressure.
- FIG. 6. Cells showing lines of gliding growth, in roots under lateral pressure.
- FIG. 7. Gliding growth of cells, under pressure, as at *a*.
- FIG. 8. Longitudinal section of root, showing manner of growth of root cells under pressure.
- FIG. 9. Longitudinal section of root, in which nuclear and cell divisions have continued until a large number of closely packed cells have been produced. Mitotic figures normal in position but few in number.
- FIG. 10. Moniliform root of *Vicia faba*, grown in moist air, and dipped into cold water for an instant every day.



THE INFLUENCE OF BORON ON THE CHEMICAL COMPOSITION AND GROWTH OF THE TOMATO PLANT

EARL S. JOHNSTON AND W. H. DORE

(WITH NINE FIGURES)

Introduction

An investigation was undertaken by one of the writers for the purpose of determining the minimum potassium requirements of the tomato plant. It was planned to use water cultures in these studies. Although the solutions contained the so-called essential elements for normal plant growth it was soon discovered that the plants failed to grow. Since attention had been recently called to the importance of boron and manganese it was thought that a possible solution to the problem lay in adding these elements to the culture medium and a special experiment was conducted to test their effects on the growth of the tomato. Manganese (1.0 ppm.) was added to the nutrient solution as manganese sulphate and boron (0.55 ppm.) as boric acid. The four groups noted in table I consisted of nine cultures, each containing a single plant in a two-quart Mason jar.

TABLE I

DATA SHOWING AVERAGE HEIGHT AND DRY WEIGHT OF TOMATO PLANTS GROWN IN
BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS WITH 0.55 PPM.
BORON AND WITH 1.0 PPM. MANGANESE

GROUP	SOLUTION	HEIGHT	DRY WEIGHT		
			TOPS	ROOTS	TOTAL
		<i>cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
A	No B and Mn ..	24.4	3.0	0.3	3.3
B	B and Mn added	55.2	6.4	1.5	7.9
C	Mn added .	24.3	3.0	0.3	3.3
D	B added .	55.2	5.4	1.2	6.6

An inspection of this table at once shows that the plants of cultures containing boron are far more normal than the others. It cannot, however, be concluded that manganese is not essential. Although chemicals of a good grade were used in these experiments there is no assurance that they were completely free from manganese and boron. Nevertheless, the experiment does show that the tomato plant requires an appreciable amount of boron over and above that present as impurities in the solutions used. Figure 1 shows very clearly the differences in growth of two representative

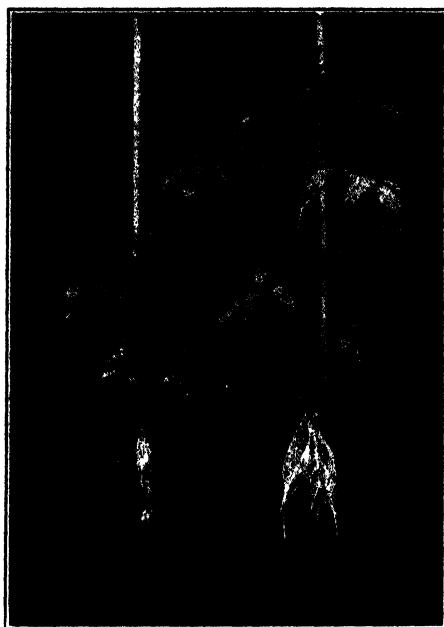


FIG. 1. Photograph showing boron deficient tomato plant (left) and one grown in a similar solution to which 0.55 ppm. boron as boric acid had been added (right).

plants of this experiment, one (left) from a culture deficient in boron, the other (right) from a similar solution to which boron (0.55 ppm.) as boric acid had been added.

The growth responses of the tomato to boron were so marked and interesting that a more detailed study was undertaken. The necessity of boron to the growth of other plants has been pointed out by other investigators within the last few years. The earlier workers with boron concerned themselves with detecting its presence in various plants. The amounts found as boric acid varied considerably, sometimes running as high as one per cent. or slightly above. The presence of boron *per se* in the plant is, however, no indication that it is essential for growth. The natural supposition would be that its presence is a detriment rather than an asset, since very small amounts are known to be exceedingly toxic. For reviews of the literature and descriptions of experiments indicating the necessity of boron for normal plant growth and development the reader is referred to BRENCLEY (2), BRENCLEY and WARINGTON (3), SOMMER and LIPMAN (14), SWANBACK (15), and WARINGTON (16). In a preliminary paper JOHNSTON and DORE (8) called attention to the necessity of boron for growth of the tomato plant.

Experimentation

The experiments described in this paper were carried out in the Division of Plant Nutrition at the University of California and in the Department of Plant Physiology at the University of Maryland. The variety of tomato used at California was the Santa Clara Canner, and at Maryland this same variety and Marglobe were used. The seeds were germinated between layers of moist filter-paper. When the roots were 2 to 10 mm. long the young plants were transferred to a germination net similar to that described by JOHNSTON (7). After the seedlings had reached approximately 2 to 3 cm. in length they were transferred to the culture solutions. Each culture consisted of a single plant supported by means of a little cotton in a paraffined flat cork stopper which fitted into a two-quart Mason jar containing the nutrient solution. The jars were wrapped with heavy paper to exclude most of the light from the roots.

The general nutrient solutions used in the California experiments were made up from the following salts: calcium nitrate, magnesium sulphate, magnesium phosphate (primary and secondary), potassium sulphate, manganese sulphate and ferric tartrate. The approximate calculated concentrations of the usual ions in this general nutrient solution expressed as parts per million and milliequivalents were:

	ppm.	milliequivalents		ppm.	milliequivalents
Ca	200	10.0	NO ₃	620	10.0
Mg	60	4.9	SO ₄	290	6.0
K	78	2.0	PO ₄	74	2.3
Fe (enough to keep plants green)			Mn	1	0.0364

In the Maryland experiments the general nutrient solution was made up from salts specially prepared by J. T. Baker Chemical Co. for the Committee on Salt Requirements of the National Research Council, the manganese sulphate and ferric tartrate were of equally high grade but obtained from other sources. The Maryland solutions of slightly different composition had the following partial volume molecular concentrations:

Ca(NO ₃) ₂	0.005
MgSO ₄	0.002
KH ₂ PO ₄	0.002
MnSO ₄	0.0000178

To these general nutrient solutions ferric tartrate (0.5 per cent. solution) was added at the rate of a cubic centimeter per liter per day while the plants were young. After the roots were well developed iron was added less frequently. Boron was added as boric acid to the cultures so designated. Expressed as parts per million and milli-equivalents the ions of the

basic solution used in the Maryland experiments had the following approximate values:

	ppm.	milliequivalents		ppm.	milliequivalents
Ca	200	10.0	NO ₃	620	10.0
Mg	49	4.0	SO ₄	194	4.0
K	78	2.0	PO ₄	190	6.0
Fe (enough to keep plants green)			Mn	1	0.0364

A second preliminary experiment was conducted to determine an approximate range of tolerance of the tomato plant toward boron. Four sets of cultures were set up on October 29, 1926. Each set consisted of 10 two-quart culture jars with a single plant per jar. To the general nutrient solutions boron was added at the following rates:

Group A	0.000 ppm.
Group B	0.055 ppm.
Group C	0.550 ppm.
Group D	5.500 ppm.

The plants were harvested on December 10. The average length of stem and dry weight per plant as well as the approximate total transpiration are given in table II.

TABLE II

DATA SHOWING AVERAGE HEIGHT AND DRY WEIGHT OF TOMATO PLANTS, TOGETHER WITH THEIR APPROXIMATE TOTAL TRANSPIRATION WHEN GROWN IN SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF BORON

GROUP	BORON	STEM HEIGHT	DRY WEIGHT			TRANSPI- RATION
			TOPS	ROOTS	TOTAL	
	<i>ppm.</i>	<i>cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>cc.</i>
A	0.000	35.1	2.66	0.22	2.88	952
B	0.055	53.4	3.69	0.62	4.31	1584
C	0.550	54.3	3.63	0.63	4.26	1562
D	5.500	45.8	2.75	0.40	3.15	1152

Weekly measurements of stem height were made, but only the final average for each group is included in the table. While making the height measurements on December 2 it was observed that the stems and petioles of the plants in group A were exceedingly brittle. In later experiments it was found that this brittleness was characteristic of tomato plants grown in boron deficient solutions. In fact, the brittleness usually occurred before any visible manifestation of boron deficiency appeared. The brittleness of stems and petioles associated with boron deficiency is best described as similar to the breaking of a piece of cheese and not that characteristic of

turgid tissues, which usually break with a snap. The data in table II bring out quite clearly an approximate range of boron concentration, below and above which growth is inhibited. Plants of group A, to which no boron was added, showed a decided retardation of growth and distinct injuries. Another marked symptom of boron deficiency is the dying of the growing point of the stem. In a short time this tissue becomes blackened and dried, thus terminating stem elongation. The stem height measurements of table II bring this out as well as figure 1. Plants of group D, to which 5.5 ppm. of boron were added likewise showed a retardation of growth. These plants also showed distinct injury which, however, was unlike that occurring in the boron deficient plants. With this amount of boron the leaves died at the margins. A representative leaf from a plant of this group together with a leaf from a healthy plant is shown in figure 2. No marked differ-



Fig. 2. Photograph showing boron injury to the leaf of a tomato plant (left) when grown in a nutrient solution containing 5.5 ppm. boron as boric acid. Normal leaf (right) does not show the dried and dead margins.

ences in growth were noted between the plants of groups B and C so that it might be concluded tentatively that boron in these particular culture solutions was equally good for growth of the tomato plant at concentrations of 0.055 and 0.55 ppm. It must be remembered that while 0.055 ppm. of boron may be sufficient for normal growth under one set of conditions it may be insufficient under others.

Immediately following the second experiment an investigation was started with the object of determining the chemical differences between

normal tomato plants and those grown in boron deficient media. Tomato seeds (Santa Clara Canner) were germinated in a manner already described and on December 15, 1926, the seedlings were set out in the culture jars. Two sets of cultures were used in this experiment, one, group A, without boron, except for traces that might have been present as impurities in the culture solutions, and another, group B, to which boron (0.55 ppm.) was added as boric acid. This experiment, as well as the two preliminary ones, was carried out at Berkeley, California. Each group contained 40 plants, four of which were used in preliminary microscopical tests; the remaining 36 plants of each group were harvested on February 7, 1927. The nutrient solutions were not renewed during the entire growing period. In these experiments the amount of mineral elements contained in the solutions was sufficient for good vegetative growth over the periods studied. During certain seasons in other experiments when transpiration rates were higher it was necessary to add distilled water to replace that lost, but this was not necessary in the present case.

Because of the changes in the various constituents (particularly in the amount and kind of carbohydrates) that are taking place in plant tissues during the day, special care was exercised in harvesting the plants in order that the two groups might be as comparable as possible. Six plants from each group were alternately harvested. Considerable time was consumed in this operation and without a doubt plants harvested in the morning differed considerably from those harvested in late afternoon. For this reason it seems advisable to include a table showing the time of day as well as the character of the sky when each set of six plants from the two groups was harvested. These data are presented in table III.

TABLE III

DATA SHOWING ORDER AND CONDITIONS OF HARVESTING TOMATO PLANTS

GROUP	CULTURE NUMBERS	TIME OF HARVEST		CHARACTER OF SKY
		BEGINNING	ENDING	
A and B	1- 6	9:53 a.m.	10:37 a.m.	Bright and dull
A and B	7-12	11:42 "	12:18 p.m.	Bright
A and B	13-18	2:22 p.m.	2:51 "	Bright
A and B	19-24	3:50 "	4:20 "	Bright
A and B	25-30	5:14 "	5:36 "	Clear sunset
A and B	31-36	7:30 "	7:54 "	Dark

Plants (7 to 30) of group B, which were harvested during the warmest and brightest part of the day, were in a wilted condition. The other plants

of group B were turgid when harvested. None of the plants in group A (those deficient in boron) were severely wilted at any time of the day. Plants numbered 10 and 27 in group A, and 3 and 14 in group B differed somewhat in their forms of growth from the other plants. Such variations are to be expected with a variety like the Santa Clara Canner which is highly heterogenous as noted by LESLEY and ROSA (9). Despite these variations in growth, injuries were apparent in all of the boron deficient plants.

Analytical methods

PRELIMINARY PREPARATIONS

Preparation of samples.—The harvesting operations consisted of dissecting the plants into leaves, stems and roots. An attempt was made to pull the expanded leaf tissue from the mid-rib. The petiole and often a small portion of the mid-rib were thus included with the stem tissue. The roots were severed in a plane just above the position where they branched out freely from the base of the stem. Without removing the plants from their culture jars the leaves were severed, weighed and dropped into boiling 95 per cent. alcohol. The stems were then cut from the roots and treated similarly, and finally the roots received the same treatment. The boiling process was continued for approximately 15 minutes after the plant tissues had been introduced. The flasks containing the plants and alcohol were then stoppered with paraffined cork stoppers. On cooling, the softened paraffin hardened, automatically sealing the flasks. The material was thus effectively preserved for analysis.

Separation of the sample into alcohol-soluble and alcohol-insoluble matter.—The contents of each flask were filtered and each filtrate was collected in a 1-liter graduated flask. The undissolved portion was placed in Soxhlet extraction tubes and extracted with 85 per cent. alcohol until colorless. The green solution resulting from the extraction was concentrated and added to the original filtrate. The combined alcoholic solution was then made up to volume.

Determination of the alcohol-insoluble dry matter.—The colorless insoluble residue was placed in tared weighing bottles, dried in an oven at 100° C. and weighed. The difference in weight was recorded as *alcohol-insoluble matter*. The material so obtained was used for the determination of *starch, hemicelluloses, galacturonic acid, lignin-suberin and cellulose*.

Determination of alcohol-soluble dry matter.—A 25-cc. aliquot portion was taken from each of the liter flasks and total solids were determined by weighing after exaporating in a tared porcelain dish and drying in an oven at 100° C. From the weight of solids thus obtained, the weight of *alcohol-soluble dry matter* in the liter of solution was calculated by multiplying by 40.

Determination of total dry matter.—The value representing the total dry matter was obtained by adding together the values of the *alcohol-soluble* and *alcohol-insoluble dry matter*. Water was determined by subtracting this sum from the corresponding green weight of the tissue.

ANALYSIS OF THE ALCOHOL-SOLUBLE PORTION

Preparation for the determination of sugars.—100-cc. portions of the alcoholic solutions were placed in Erlenmeyer flasks and evaporated to dryness on the steam bath with the steam turned low so as to avoid undue heating of the sugars. To remove chlorophyll and other substances preparatory to determining the sugars, the dried residue was digested three times with benzene on the steam bath, the benzene solution being poured through a folded filter. The combined benzene filtrates were evaporated in a tared beaker, dried, weighed and recorded as *benzene-soluble matter*.

One portion of the benzene-insoluble residue was in the original flask and another on the folded filter. Both were heated over the radiator to expel adhering benzene. 15 cc. of hot water were next added to the flask and, after a few minutes, when the sugars had dissolved, the solution was poured through the filter-paper dissolving the rest of the residue. Flask and filter were washed with a few cubic centimeters of hot water collecting the solution in a 110-cc. flask.

The filtrate was cooled to room temperature and basic lead acetate added drop by drop until no further precipitation occurred. The excess lead was removed by adding saturated sodium oxalate solution in slight excess. The solution was then made up to 110 cc. and unless the analysis was to be completed at once about 1 cc. of toluol was added to the solution as a preservative.

Reducing sugars.—Reducing sugars were determined on 25-cc. aliquot portions of the prepared solution by a slight modification of the Munson and Walker method, (C. A. BROWNE, Handbook of Sugar Analysis, p. 426, New York, John Wiley and Sons, 1912.) The precipitated cuprous oxide was collected on tared asbestos Gooch crucibles and washed with water in the usual manner, but instead of drying and weighing as cuprous oxide, the precipitates were ignited in a muffle furnace to cupric oxide and weighed as such. The weight of cupric oxide was multiplied by the factor 0.9 to give the weight of cuprous oxide and the corresponding weight of glucose was then obtained from Munson and Walker's tables.

Reducing sugars after inversion.—55 cc. of the prepared solution were placed in a 110-cc. flask, and 5.5 gm. of solid citric acid added. The mixture was then boiled for 10 minutes. After cooling to room temperature the solution was made alkaline to phenolphthalein by adding NaOH solution

and thereafter dilute acetic acid was added until the red color disappeared. The solution was made up to 110 cc. and reducing sugars determined on 50-cc. portions in the same manner as before.

Sucrose.—Sucrose is obtained by difference between reducing sugars before and after inversion.

ANALYSIS OF THE ALCOHOL-INSOLUBLE PORTION

Starch.—To 1 gram of the alcohol-insoluble material 100 cc. of water were added, after which the mixture was boiled for 15 minutes to gelatinize the starch. After cooling to room temperature, 0.1 gram of takadiastase and 10 drops of toluol were added. The mixture was placed in the incubator room at 28° C. and left over night. After removing the solution from the incubator room it was boiled for 15 minutes to inactivate the enzyme and then filtered through a Gooch crucible containing a disk of mercerized cotton cloth as the filtering medium. The undissolved residue was saved for the *hemicellulose* determination. The filtrate was transferred to a 200-cc. volumetric flask, clarified with basic lead acetate, delead with sodium oxalate solution and made up to volume. The mixture was filtered through a dry filter and 50 cc. of the filtrate were mixed with 5 cc. of concentrated hydrochloric acid and boiled for 2.5 hours under a reflux condenser. After cooling to room temperature the solution was made alkaline with sodium hydroxide solution, then slightly acid with dilute acetic acid. It was then made up to volume and reducing sugars expressed as glucose. Starch was calculated by multiplying by the factor 0.9.

Hemicelluloses.—The residue remaining after the takadiastase digestion was transferred to an Erlenmeyer flask with a measured volume of water. To this were added 8 cc. of 12 per cent. hydrochloric acid and water to make a total volume of 100 cc. The mixture was heated on the hot plate under a reflux condenser for 2.5 hours. The insoluble residue was then filtered off and washed and the filtrate transferred to a 200-cc. volumetric flask. After cooling to room temperature the solution was made alkaline with NaOH, then slightly acid with acetic acid. It was clarified by adding basic lead acetate in slight excess followed by sodium oxalate solution to remove the excess lead. The solution was made up to 200 cc., mixed, filtered through a dry filter, and reducing sugars determined in 50-cc. portions. The results were calculated to the hexosan formula by multiplying the glucose value by 0.9.

Residue after hemicelluloses.—The insoluble residue remaining after the hydrolysis of the hemicelluloses was collected on a Gooch crucible containing a filtering disk of mercerized cotton cloth, dried in the oven at 100° C. and weighed.

Lignin-suberin.—The dried residue after hydrolysis of hemicelluloses was digested for 24 hours in 20 cc. of 72 per cent. sulphuric acid. The mixture was then diluted with 300 cc. of water, heated to boiling, filtered on a Gooch crucible with a cloth filter disk, washed, dried and weighed.

Cellulose by loss.—The material soluble in the 72 per cent. sulphuric acid was regarded as cellulose. The value was obtained by subtracting the *lignin-suberin* value from the *residue after hemicelluloses*.

Galacturonic acid.—Galacturonic acid was determined by DORE's (5) modification of the Lefevre method. One gram of the alcohol-insoluble dry material was placed in a liter flask with 100 cc. of 12 per cent. hydrochloric acid. The flask was placed in a carbon dioxide absorption train and, after sweeping out the carbon dioxide due to inorganic carbonates and that previously present in the flask by a current of carbon-dioxide-free air, the decarboxylation reaction was carried out by heating the flask in an oil bath at 130° C. The carbon dioxide produced by this reaction was swept out of the flask and absorbed in a weighed Geissler potash bulb, suitable precautions being taken to prevent other reaction products from passing over. The reaction was continued until the Geissler bulb showed constant weight, which usually required 4.5 hours. The weight of carbon dioxide multiplied by 4 gives the weight of hexuronic acid present in the plant tissue. Since the galacturonic acid of pectic substances is the only hexuronic acid definitely known to occur extensively in plant tissues, it is assumed that the hexuronic acid value is identical with the galacturonic acid content. This in turn is a measure of the pectic substances, but since the factor is variable, no attempt has been made to express the results in terms of pectic substance.

Chemical data

The data presented in table IV give in detail the green weights of leaves, stems and roots of individual plants. As is to be expected, considerable variation in growth occurs, but in general the plants in each group were fairly uniform. The average total green weights of the subgroups of group A fluctuate with the time of day at which the samples were obtained. Reference to table III will show this. These average values drop from 41.6 in the first period to 36.9 in the third, then rise to 40.1 in the last. Such variations in green weight are to be expected as the water content of the plants varies with a change in atmospheric conditions throughout the day. The low value of 36.9 in the third period is, however, due to the low value of the leaves of plant numbered 18. Several leaves from this plant had been removed several days previous to the date of harvest and their weights are not included in the table. If the other five values are averaged then 36.9 becomes 38.4. The third, fourth and fifth

subgroups then become practically equal with reference to their total green weights. The subgroup values of group B do not show any regular decrease or increase in the total green weight. With the exception of the fourth period the values are quite uniform. These general observations suggest the possibility that plants of group B are slightly better able to maintain a constant moisture content in spite of the fluctuating condition of their environment which tends to bring about a water deficit of the tissues, while plants of group A seem to lack this ability. Such a general inspection of the data would indicate a somewhat greater state of saturation deficit occurring in the boron deficient plants during the middle of the day than was the case under like conditions in the normal plants grown under similar environmental conditions. This, however, is not the case as will be seen from the discussion of table VI.

The amounts of alcohol-insoluble and alcohol-soluble dry matter in plants grown in boron deficient solutions and in solutions to which boron was added are presented in table V. For convenience this table is divided into three sections, l, s, and r, representing respectively data from leaves, stems and roots. In part V—l it will be noted that no significant difference was found between the average total dry weight per plant in group A and that in group B, the latter being slightly greater, 0.27 gram, or about 14 per cent. In each group the percentage of alcohol-insoluble dry matter is approximately twice that of the alcohol-soluble material. The alcohol-insoluble matter is 7 per cent. less in the boron deficient plants than in the normal plants while the alcohol-soluble dry matter is correspondingly increased. A survey of these values for the plant stems in part V—s shows an increase of approximately 60 per cent. in dry matter of the normal plants over the boron deficient ones, these values being 2.83 and 1.76 grams respectively. While the actual difference between the two groups is significant, the percentage of alcohol-insoluble and alcohol-soluble matter of the total dry weight is the same. Part V—r clearly shows the greater average total dry matter in the roots of group B plants. Here the increase is over 200 per cent., by far the greatest of measurable quantities noted in these experiments. The relative proportion of alcohol insoluble and soluble matter in the two groups is practically the same.

So far as the data of table V go, it appears that no marked difference in chemical composition of roots and stems is to be expected between plants of the boron deficient group and those grown in a medium containing boron. There is, however, indication of a difference in chemical composition of the leaves from plants in these two groups. The leaves of the boron deficient plants contain a higher percentage of alcohol-soluble matter than do the normal leaves. Since many of the metabolically important constituents

TABLE IV

DATA SHOWING GREEN WEIGHTS OF LEAVES, STEMS, ROOTS AND THE TOTAL WEIGHT OF INDIVIDUAL PLANTS GROWN IN BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON (0.55 PPM.) WAS ADDED

No.	GROUP A (BORON DEFICIENT)				GROUP B (BORON—0.55 PPM.)			
	Leaves	Stems	Roots	Total	Leaves	Stems	Roots	Total
1	15.0	24.0	4.3	43.3	15.6	32.5	10.9	59.0
2	12.9	22.4	2.8	38.1	16.2	33.9	14.2	64.3
3	14.6	22.6	3.7	40.9	16.8	31.0	15.2	63.0
4	16.2	20.8	3.0	40.0	13.9	30.3	11.1	55.3
5	15.5	22.6	3.4	41.5	15.3	35.8	11.5	62.6
6	17.8	24.8	3.1	45.7	16.2	32.7	13.6	62.5
Total ...	92.0	137.2	20.3	249.5	94.0	196.2	76.5	366.7
Average ...	15.3	22.9	3.4	41.6	15.7	32.7	12.8	61.1
7	14.6	22.6	3.1	40.3	15.3	33.7	12.9	61.9
8	14.9	22.9	3.4	41.2	16.5	31.7	14.2	62.4
9	16.7	22.0	2.9	41.6	16.1	33.0	17.3	66.4
10	14.1	19.9	3.2	37.2	15.4	34.2	12.3	61.9
11	14.1	21.2	2.8	38.1	14.3	31.2	11.9	57.4
12	12.6	21.1	3.4	37.1	13.6	30.6	12.9	57.1
Total .	87.0	129.7	18.8	235.5	91.2	194.4	81.5	367.1
Average ..	14.5	21.6	3.1	39.3	15.2	32.4	13.6	61.2
13	14.5	20.7	3.4	38.6	16.2	34.7	17.1	68.0
14	15.8	18.4	2.8	37.0	18.1	32.4	16.8	67.3
15	14.6	20.6	3.8	39.0	15.2	31.6	13.0	59.8
16	12.4	21.7	3.5	37.6	15.2	30.3	12.7	58.2
17	13.9	22.5	3.4	39.8	16.6	28.8	16.3	61.7
18	6.4	20.0	3.2	29.6	13.3	30.5	10.2	54.0
Total	77.6	123.9	20.1	221.6	94.6	188.3	86.1	369.0
Average	12.9	20.7	3.4	36.9	15.8	31.4	14.4	61.5
19	15.6	23.1	2.8	41.5	16.7	35.0	12.6	64.3
20	12.9	20.6	3.5	37.0	15.9	35.4	13.3	64.6
21	12.6	21.0	3.2	36.8	17.4	41.7	14.1	73.2
22	13.0	19.3	3.3	35.6	15.8	34.3	14.4	64.5
23	14.3	22.7	3.6	40.6	17.3	35.3	14.6	67.2
24	17.4	20.6	3.1	41.1	16.9	36.7	14.8	68.4
Total .	85.8	127.3	19.5	232.6	100.0	218.4	83.8	402.2
Average	14.3	21.2	3.3	38.8	16.7	36.4	14.0	67.0
25	15.3	20.3	3.7	39.3	13.8	28.4	9.0	51.2
26	12.6	21.9	3.0	37.5	16.8	34.6	14.1	65.5
27	12.5	21.6	3.1	37.2	16.6	34.1	13.5	64.2
28	15.6	21.6	2.4	39.6	16.9	35.5	14.8	67.2
29	13.4	21.1	3.0	37.5	17.2	35.8	13.7	66.7
30	13.8	21.6	3.7	39.1	16.0	32.3	12.0	60.3
Total . .	83.2	128.1	18.9	230.2	97.3	200.7	77.1	375.1
Average . . .	13.9	21.4	3.2	38.4	16.2	33.5	12.9	62.5
31	13.6	22.5	3.6	39.7	15.7	39.4	11.6	66.7
32	15.0	22.8	3.8	41.6	15.0	33.7	11.8	60.5
33	11.3	19.8	3.2	34.3	16.9	35.6	12.3	64.8
34	15.4	20.5	3.6	39.5	17.1	33.5	11.4	62.0
35	16.6	23.5	3.3	43.4	14.2	35.0	9.3	58.5
36	16.0	23.2	2.9	42.1	16.3	30.2	9.9	56.4
Total .. .	87.9	132.3	20.4	240.6	95.2	207.4	66.3	368.9
Average . . .	14.7	22.1	3.4	40.1	15.9	34.6	11.1	61.5
Grand total ...	513.5	778.5	118.0	1410.0	572.3	1205.4	471.3	2249.0
Grand average per plant	14.3	21.6	3.3	39.2	15.9	33.5	13.1	62.5

TABLE V—1

DATA SHOWING AMOUNT OF DRY MATTER IN *leaves* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		TOTAL
	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>
1- 6	7.62	64	4.32	36	11.94
7-12	7.22	62	4.41	38	11.63
13-18	6.72	*	*	*	*
19-24	6.96	61	4.39	39	11.35
25-30	6.68	61	4.27	39	10.95
31-36	7.06	61	4.57	39	11.63
Average per plant	1.17	62	0.73	38	1.91
Group B (boron 0.55 ppm.)					
1- 6	8.40	69	3.72	31	12.12
7-12	8.93	70	3.74	30	12.67
13-18	9.11	69	4.12	31	13.23
19-24	9.84	69	4.49	31	14.34
25-30	9.41	70	3.96	30	13.37
31-36	8.82	69	3.96	31	12.78
Average per plant	1.51	69	0.67	31	2.18

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask.

TABLE V—s

DATA SHOWING AMOUNT OF DRY MATTER IN *stems* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		ORIGINALLY ALCOHOL SOLUBLE BUT SUBSEQUENTLY PRECIPITATED		TOTAL
	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>
1-18	21.32	65.0	11.26	34.4	0.21	0.6	32.79
19-36	19.99	65.7	10.34	34.0	0.08	0.3	30.41
Average per plant	1.15	65.35	0.60	34.2	0.01	0.45	1.76
Group B (boron 0.55 ppm.)							
1-18	32.18	66.4	15.84	32.7	0.45	0.9	48.47
19-36	34.61	64.7	18.41	34.4	0.45	0.8	53.47
Average per plant	1.86	65.5	0.95	33.6	0.02	0.9	2.83

TABLE V—r

DATA SHOWING AMOUNT OF DRY MATTER IN *roots* OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

GROUP A (BORON DEFICIENT)	ALCOHOL INSOLUBLE		ALCOHOL SOLUBLE		TOTAL
	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>	<i>per cent.</i>	<i>gm.</i>
1-18	3.96	81.5	0.90	18.5	4.86
19-36	4.17	81.9	0.92	18.1	5.09
Average per plant	0.23	81.7	0.05	18.3	0.28
Group B (boron 0.55 ppm.)					
1-18	13.29	84.2	2.50	15.8	15.79
19-36	13.41	83.3	2.68	16.7	16.09
Average per plant	0.74	83.8	0.14	16.3	0.88

of plant sap are soluble in alcohol, these results immediately suggest that there are significant differences in the active mobile constituents of the two groups of plants; while the insoluble constituents, which include the framework constituents of the plant, are much alike in the two groups. As will be shown later, this is the situation which actually exists.

In table VI data are presented which show the water content of leaves, stems and roots as related to the green and dry weights of plants grown in boron deficient solutions and in solutions containing boron. The weight measurements are expressed in grams and represent the total weight from 6 plants in the case of leaves and from 18 plants in case of stems and roots. In the discussion of table IV attention was called to an apparent decrease in water content in plants of group A. In table VI such a decrease is apparent for leaves of plants in group A harvested during the warm part of the day namely, plants numbered, 7-12, 19-24 and 25-30. However, if the water content of the leaves of each group is compared with the dry weight values, the ratio of water per unit of dry matter is found to be fairly constant throughout the day. On the other hand, these ratio values for leaves of plants in group B fluctuate quite regularly with the periods of the day during which they were harvested. It must be concluded from the data in this table that leaves of the normal tomato plants (group B) showed a tendency to lose water toward midday, while those in the boron deficient group maintained practically the same water-dry weight ratio values throughout the day. Actual observation of the plants as they were harvested bears this out. The leaves of plants 7 to 30 of group B that were collected during the middle of the day were wilted. Leaves of the other plants of this group, 1 to 6 and 31 to 36, which were collected in early

TABLE VI

DATA SHOWING THE WATER CONTENT OF LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED

CULTURES	GROUP A (BORON DEFICIENT)				GROUP B (BORON-0.55 PPM.)			
	GREEN WEIGHT	DRY WEIGHT	WATER	WATER PER UNIT DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	WATER	WATER PER UNIT DRY WEIGHT
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Leaves 1-6	92.0	11.94	80.06	6.71	94.0	12.12	81.88	6.76
7-12	87.0	11.63	75.37	6.48	91.2	12.67	78.53	6.20
13-18	*	*	*	*	94.6	13.23	81.37	6.15
19-24	85.8	11.35	74.45	6.56	100.0	14.34	85.66	5.97
25-30	83.2	10.95	72.25	6.60	97.3	13.37	83.93	6.20
31-36	87.9	11.61	76.29	6.57	95.2	12.78	82.42	6.45
Average per plant	14.5	1.91	12.61	6.57	15.9	2.18	13.72	6.29
Stems 1-18	390.8	32.79	358.01	10.92	578.9	48.47	530.43	10.94
19-36	387.7	30.41	357.29	11.75	626.5	53.47	573.03	10.72
Average per plant	21.6	1.76	19.87	11.33	33.5	2.83	30.65	10.83
Roots 1-18	59.2	4.86	54.34	11.2	244.1	15.79	228.31	14.5
19-36	58.8	5.09	53.71	10.6	227.2	16.09	211.11	13.1
Average per plant	3.28	0.28	3.00	10.9	13.09	0.88	12.21	13.8

* Results not determined because alcohol soluble material of these leaves was lost from a broken flask.

morning and late evening were not wilted. From the work of RENNER (12), LIVINGSTON and BROWN (11), LIVINGSTON (10), JOHNSTON (6) and others, such a diminution in water content is to be expected during the day in normal healthy plants. An interesting point to be mentioned is that none of the leaves from plants in group A was severely wilted at any time. On a *priori* reasoning alone it might be concluded that either the water absorbing and conducting tissues of the boron deficient plants were entirely adequate to supply water to the leaves as fast as lost by transpiration, or that the structure of the leaves was such as to greatly retard water loss.

There is but little difference to be noted in the average dry weight of leaves per plant of the two groups. A more striking difference is seen in the dry weights of the stems and roots of the plants of these groups. The average dry weight of stems of the normal plants was approximately 1.6 as great as the stems of the boron deficient plants. The most striking difference between the boron deficient plants and the normal ones occurs in the root development. Roots of the normal plants are practically three times

as great when dry weight is used as the criterion of measurement. Poor root development is apparently a definite characteristic of boron deficient plants since it has been observed in other plants by other investigators.

Table VII presents data showing the composition of alcohol-soluble matter in plants grown in boron deficient solutions and in solutions to which boron was added. These values are expressed as percentages of total dry matter in the leaves, stems and roots respectively. The leaves of the boron deficient plants have in every case a much higher content of reducing sugars and of total sugars than the corresponding normal plants. With but one exception the same relationship holds for their sucrose content. The total sugars in the leaves of the normal plants increase as the day advances to a maximum in the late afternoon. The total sugars in the leaves of the boron deficient plants likewise rise to a maximum in the late afternoon, but unlike the normal plants, the total sugars do not drop off in the early evening hours. In this respect the boron deficient plants show distinctly abnormal behavior, the significance of which will be discussed later.

The stems of the boron deficient plants have lower contents of reducing sugars and total sugars than the stems of the normal plants, the relationship being the reverse of that found in the leaves. The meaning of this will also be discussed later. The relationships in the roots are similar to those in the leaves; the boron deficient roots have much higher contents of reducing sugars, sucrose and total sugars.

Another very striking difference between the plants of the A and B groups exists in the percentages of benzene-soluble matter. This material occurs more abundantly in the leaves of the normal plants and in the stems of the boron-deficient plants. The values for the roots are practically the same. This benzene-soluble matter may include chlorophyll, fats, sterols and lipins. Unfortunately we have not found it practicable as yet to undertake a thorough study of this material.

The analytical data showing the composition of the alcohol-insoluble matter of these plants are given in table VIII. These data are likewise expressed as percentages of total dry matter for each of the plant tissues studied. Perhaps the most striking difference is the greater starch content of all the parts of the boron-deficient plants. The hemicellulose is likewise somewhat greater in the leaves of group A plants, but slightly less when the stems are compared. Galacturonic acid gives somewhat higher values for the leaves of group B plants. It is doubtful if the other values are significant. Cellulose is slightly higher in the leaves and stems of the normal plants.

The analytical data presented in tables VII and VIII have been summarized in table IX. For the leaves, the outstanding differences between

TABLE VII

DATA SHOWING COMPOSITION OF ALCOHOL-SOLUBLE MATTER IN LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED. THESE DATA ARE EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

CULTURES	GROUP A (BORON-DEFICIENT)				GROUP B (BORON—0.55 PPM.)					
	ALCO- HOL- SOLU- BLE MATTER	REDUC- ING SUGARS (HEX- OSES)	SUC- ROSE	TOTAL SUGARS	BEN- ZENE SOLU- BLE MATTER	ALCO- HOL- SOLU- BLE MATTER	REDUC- ING SUGARS (HEX- OSES)	SUC- ROSE	TOTAL SUGARS	BEN- ZENE SOLU- BLE MATTER
Leaves 1-6	36	6.84	2.16	9.00	9.36	31	2.79	1.24	4.03	13.64
7-12	38	9.12	0.76	9.88	8.74	30	4.20	0.90	5.10	13.50
13-18	*	*	*	*	*	31	3.72	1.55	5.27	12.40
19-24	39	7.41	5.46	12.87	10.14	31	3.72	2.48	6.20	12.71
25-30	39	9.36	3.51	12.87	10.14	30	4.50	0.60	5.10	12.90
31-36	39	8.19	4.68	12.87	9.75	31	4.03	1.24	5.27	13.02
Average per plant	38	8.18	3.31	11.50	9.63	31	3.83	1.34	5.16	13.03
Stems 1-18	34.4	4.21	3.29	7.50	3.42	32.7	7.62	2.42	10.04	1.57
19-36	34.0	6.43	2.96	9.39	3.35	34.4	9.77	3.37	13.14	1.91
Average per plant	34.2	5.32	3.13	8.45	3.39	33.6	8.70	2.89	11.59	1.74
Roots 1-18	18.5	2.57	1.79	4.36	4.01	15.8	0.95	1.44	2.39	4.65
19-36	18.1	1.90	2.34	4.24	4.13	16.7	1.18	1.64	2.82	4.68
Average per plant	18.3	2.24	2.07	4.30	4.07	16.3	1.07	1.54	2.61	4.67

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask.

the two groups are their sugar, benzene-soluble matter and starch content. Somewhat similar differences appear in the stems, but the order is reversed for total sugars and benzene-soluble matter. Sugars formed in the leaves are, under normal conditions, transported to the stem. In the boron-

TABLE VIII

DATA SHOWING COMPOSITION OF ALCOHOL-INSOLUBLE MATTER IN LEAVES, STEMS AND ROOTS OF PLANTS GROWN IN BORON-DEFICIENT SOLUTIONS AND IN SOLUTIONS TO WHICH BORON WAS ADDED. THESE DATA ARE EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

CULTURES	GROUP A (BORON-DEFICIENT)						GROUP B (BORON 0.55 PPM.)					
	ALCOHOL- INSOLU- BLE MATTER	STARCH	HEMI- CELLU- LOSE	GALACTU- RONIC ACID	LIGNIN SUBERIN	CELLU- LOSE	ALCOHOL- INSOLU- BLE MATTER	STARCH	HEMI- CELLU- LOSE	GALACTU- RONIC ACID	LIGNIN SUBERIN	CELLU- LOSE
Leaves												
1-6	64	12.85	3.07	8.32	1.66	11.71	69	6.02	2.10	8.14	1.76	12.49
7-12	62	12.05	2.53	6.82	1.24	9.83	70	7.17	2.27	9.94	1.58	12.95
13-18	62*	11.61	2.38	8.56	1.67	11.93	69	7.67	2.18	10.35	1.45	10.94
19-24	61	11.61	2.44	7.44	1.49	10.52	69	8.89	1.38	10.07	1.38	12.11
25-30	61	11.61	2.39	9.27	1.31	9.30	70	10.81	1.99	7.98	1.96	10.26
31-36	61	12.44	2.51	9.88	1.37	9.18	69	9.88	1.38	10.49	2.07	12.59
Average per plant	62	12.03	2.55	8.38	1.46	10.41	69	8.41	1.88	9.50	1.70	11.89
Stems												
1-18	65.0	5.80	4.78	11.05	6.05	20.57	66.4	1.27	5.74	11.22	6.01	24.60
19-36	65.7	5.04	4.65	11.69	5.55	22.08	64.7	1.63	5.95	11.00	5.08	25.43
Average per plant	65.4	5.42	4.72	11.37	5.80	21.32	65.6	1.45	5.85	11.11	5.55	25.02
Roots												
1-18	81.5	0.65	7.04	12.06	14.30	18.58	84.2	0.34	6.60	9.43	12.71	19.07
19-36	81.9	1.31	6.35	10.97	14.66	18.14	83.3	0.13	6.76	11.00	12.91	18.45
Average per plant	81.7	0.98	6.70	11.52	14.48	18.36	83.8	0.24	6.68	10.22	12.81	18.76

* Results not determined because alcohol-soluble material of these leaves was lost from a broken flask. Average of other values.

deficient plants the sugars accumulated because of the apparent inability of the conducting tissues in these plants to translocate them. These data for the stems show that in the normal plants with uninjured phloem tissue the sugars pass into the stems and being able to continue further in these stems very little is condensed to starch. On the other hand in the stems of the boron-deficient plants with their injured conducting systems, the sugars cannot be moved so rapidly and are condensed to starch.

TABLE IX

SUMMARY OF ANALYTICAL DATA OF TOMATO PLANTS EXPRESSED AS PERCENTAGES OF TOTAL DRY MATTER OF LEAVES, STEMS AND ROOTS RESPECTIVELY

AMOUNT OF BORON ADDED TO NUTRIENT SOLUTION (PPM.)	LEAVES		STEMS		ROOTS	
	A 0.00	B 0.55	A 0.00	B 0.55	A 0.00	B 0.55
Reducing sugars (hexoses)	8.18	3.83	5.32	8.70	2.24	1.07
Sucrose	3.31	1.34	3.13	2.89	2.07	1.54
Total sugars	11.50	5.16	8.45	11.59	4.30	2.61
Benzene soluble matter	9.63	13.03	3.39	1.74	4.07	4.67
Starch	12.03	8.41	5.42	1.45	0.98	0.24
Hemicellulose	2.55	1.88	4.72	5.85	6.70	6.68
Galacturonic acid	8.38	9.50	11.37	11.11	11.52	10.22
Lignin and suberin	1.46	1.70	5.80	5.55	14.48	12.81
Cellulose	10.41	11.89	21.32	25.02	18.36	18.76

A very striking characteristic of the boron-deficient tomato plants is the extreme brittleness of the petioles and mid-ribs. This brittleness is perhaps best described as similar to the breaking of a piece of cheese. It is entirely unlike the breaking of a turgid tissue or stem. Because of this peculiar characteristic, it was suspected that pectic substances were absent, or at least less abundant, in the middle lamella of the boron-deficient plants. Absence of a cementing substance between the cells might be the reason for such brittleness. At the suggestion of Prof. J. H. Priestley, microscopic examinations were made using the customary pectin stains but no distinct differences could be observed between sections from normal and from boron-deficient plants. The galacturonic acid determinations given in table VIII are not significantly different for the two groups, and when these figures are calculated to the basis of alcohol-insoluble dry matter (a more comparable basis, since the fluctuating soluble constituents are eliminated) the difference between the two groups is so slight as to fall within the experimental error of the determination. It is apparent that neither the micro-chemical observations nor the galacturonic acid determinations support the

theory that brittleness is due to a deficiency of pectic material. A special experiment was, therefore carried out for the purpose of obtaining enough leaf material for pectic analysis.

This experiment was made at the University of Maryland. On November 1, 1927, young tomato seedlings were set out in two-quart culture jars. The cultures were divided into three groups, a single plant per culture. All the solutions were similar with the exception of their boron content. To group A no boron was added. Groups B and C contained 0.011 and 0.55 ppm. respectively of boron as boric acid. At the end of six weeks the leaves were cut from the plants at the base of the leaf blade. It is unfortunate that the petioles were not included in the samples, but since the mid-rib shows the same characteristic brittleness as the petioles, not a great deal of additional information would have been obtained by including the petioles. The pectic materials¹ were determined by the general methods described by CONRAD (4) and by APPLEMAN and CONRAD (1). The data are presented in table X.

TABLE X

DATA SHOWING RELATION OF BORON TO PECTIC MATERIALS IN TOMATO LEAVES EXPRESSED AS PERCENTAGE OF THEIR DRY WEIGHT

AMOUNT OF BORON ADDED TO NUTRIENT SOLUTION (PPM.)	0.00	0.011	0.55
Pectin	0.00	0.00	0.00
Pectic acid and pectates . . .	0.00	0.00	0.00
Protopectin	6.98	6.54	5.85
Reducing sugar	5.42	4.51	4.11
Sucrose	0.22	0.31	0.50
Total sugar	5.64	4.82	4.61

So far as pectin, pectic acid and pectates are concerned no measurable quantities were found in any of the groups, even though the boron deficient plants were characteristically brittle. The leaves of plants supplied with 0.55 ppm. boron showed somewhat less protopectin than the leaves of group A. It must be concluded from the evidence here presented that the brittleness is due to something other than pectic materials. It is interesting to note that the total sugar analyses follow in general the results obtained in the California experiments.

Growth data

The first boron experiment conducted at Maryland was outlined for the purpose of comparing the growth of two different varieties of tomato plants

¹ The analyses in this experiment were made by Dr. C. M. Conrad.

in water cultures of three concentrations of boron, 0.0, 0.011 and 0.55 ppm. Each of the six groups was composed of 20 cultures of one plant each. The experiment covered the period from September 10 to October 22, 1927. The average data per plant of each group are presented in table XI.

TABLE XI

DATA SHOWING WEEKLY HEIGHT (CM.), TOTAL TRANSPIRATION (CC.) AND GREEN AND DRY WEIGHTS (GM.) EXPRESSED AS THE AVERAGE PER PLANT PER EACH GROUP

WEEK ENDING 1927	PLANT GROUPS					
	A	a	B	b	C	c
	cm.	cm.	cm.	cm.	cm.	cm.
September 17 ..	2.5	2.8	3.1	3.3	3.0	3.3
24 ..	4.2	4.4	6.5	6.7	6.3	6.8
October 1 ..	7.4	5.8	14.3	13.2	14.2	13.7
8 ..	13.0	10.0	28.1	24.6	29.2	26.0
15 ..	17.3	13.3	29.2	27.1	35.8	31.6
22 ..	19.6	17.0	29.3	27.5	41.4	36.5
	cc.	cc.	cc.	cc.	cc.	cc.
Transpiration	380	253	1565	1389	2085	2033
	gm.	gm.	gm.	gm.	gm.	gm.
Green weight						
Tops	15.6	11.6	43.8	40.5	50.9	48.6
Roots ..	2.7	2.3	10.7	10.6	18.9	19.4
Total ..	18.3	13.9	54.5	51.1	69.8	68.0
Dry weight						
Tops ..	1.32	0.95	4.20	3.89	4.79	4.29
Roots ..	0.14	0.11	0.69	0.67	1.35	1.31
Total ..	1.46	1.06	4.89	4.56	6.14	5.60

Note: A, B, C; Santa Clara Canner; a, b, c, Marglobe.

A, a, No boron added to solutions.

B, b, 0.011 ppm. boron added as boric acid.

C, c, 0.55 ppm. boron added as boric acid.

The first boron-deficiency signs were noted in groups A and a on September 19, only nine days after the seedlings were set out in the culture jars. Four days later the cotyledons and leaves were a distinct purple in color. In all probability this color was due to anthocyan, which is frequently associated with an excess sugar accumulation. Such an excess was found in the California experiments and later to a less degree at Maryland. The indications were that the conducting tissues were either destroyed or never properly developed in these plants.

The height measurements at the end of the first week indicate some injury to plants of groups A and a, and by the end of the second week little

doubt can be held as to the falling off in their rates of stem elongation. In groups A and a the terminal shoot soon died. This brought about a very peculiar form of growth. One, and often several lateral shoots developed in a manner illustrated in figures 3 and 4. The dead terminal shoot is clearly seen in figure 3. Attention is also directed to the swollen condition



FIG. 3. Photograph showing a boron-deficient tomato plant, with dead terminal shoot and lateral shoot greatly thickened at its base.

of the new shoot at a point just above the place where it joins the main stem. It would appear as if reserve food materials were accumulating at this point because of the inability of the conducting systems to transport them down the main stem.

A comparison of the two varieties shows Santa Clara Canner somewhat superior to Marglobe when stem height, and green and dry weights are used as growth criteria. Each variety was equally susceptible to boron-deficiency injuries and for such studies either one may be used. The data

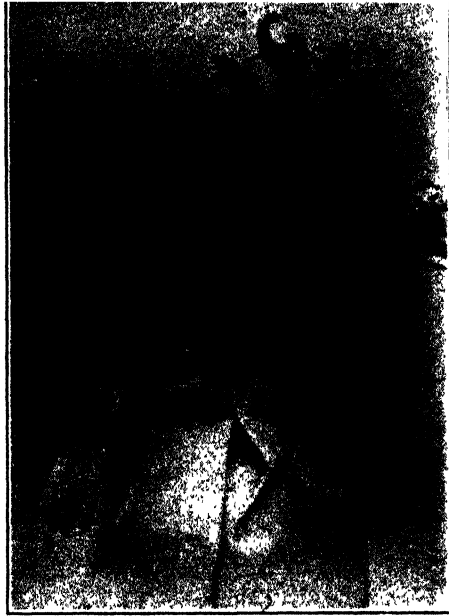


FIG. 4. Photograph showing a boron-deficient tomato plant in which a number of new shoots have arisen below the dead terminal shoot.

of table XI show another interesting point. The values for groups B and b at the end of the experiment are between those for groups A, a and C, c in every case. Indications are that boron has a quantitative effect. With 0.011 ppm. boron in the nutrient solution, growth could take place at a rate similar to that where 0.55 ppm. was used, but only up to a certain point. From this point the inadequate supply of boron became the limiting factor. This is quite clearly shown in the weekly height measurements. Practically no difference in height existed on October 1 between groups B and C, and b and c. One week later, B and b plants showed signs of retarded growth and by October 22 there was no question as to their inferiority when compared with the plants of groups C and c. This difference in stem heights is shown in figure 5. In a similar experiment carried out a little later in the year under slightly less favorable growing conditions practically the same results were found. Representative plants from the three groups in this experiment (boron added to solutions were 0.0, 0.011,



FIG. 5. Photograph showing tomato plants growing in similar solutions with the exception of the boron concentration. Left, without boron; center, 0.011 ppm. boron; right, 0.55 ppm. boron.

0.55 ppm.) are shown in figure 6. The growth curves of groups A, B, C, for which data appear in table XI are presented in figure 7 and perhaps

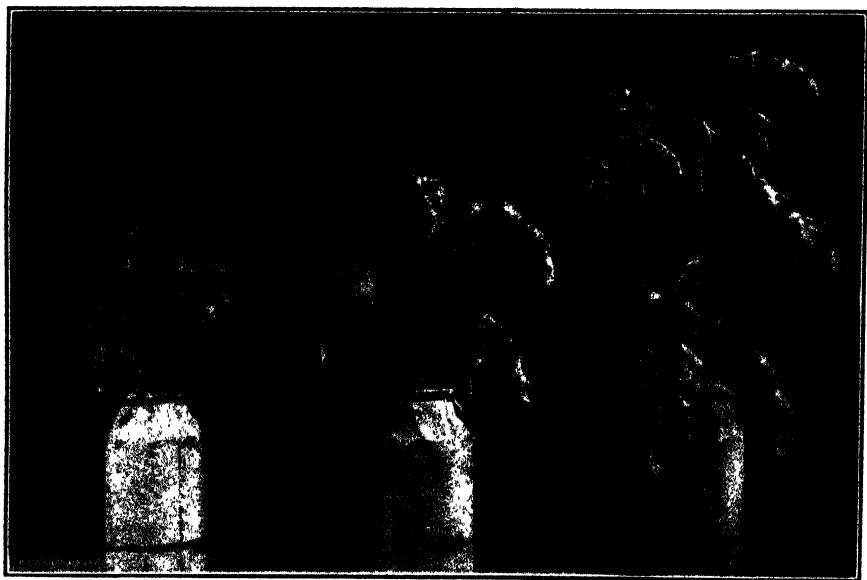


FIG. 6. Photograph showing tomato plants growing in similar solutions with the exception of the boron concentration. Left, without boron; center, 0.011 ppm. boron; right, 0.55 ppm. boron.

show to a better advantage the quantitative effect of boron on stem elongation.

Another experiment was carried out with the purpose of growing plants for a period of 6 weeks in a solution supplied with boron, then changing to a boron-deficient solution, and also, to grow other plants in a boron-deficient solution and then change to a solution containing boron. This

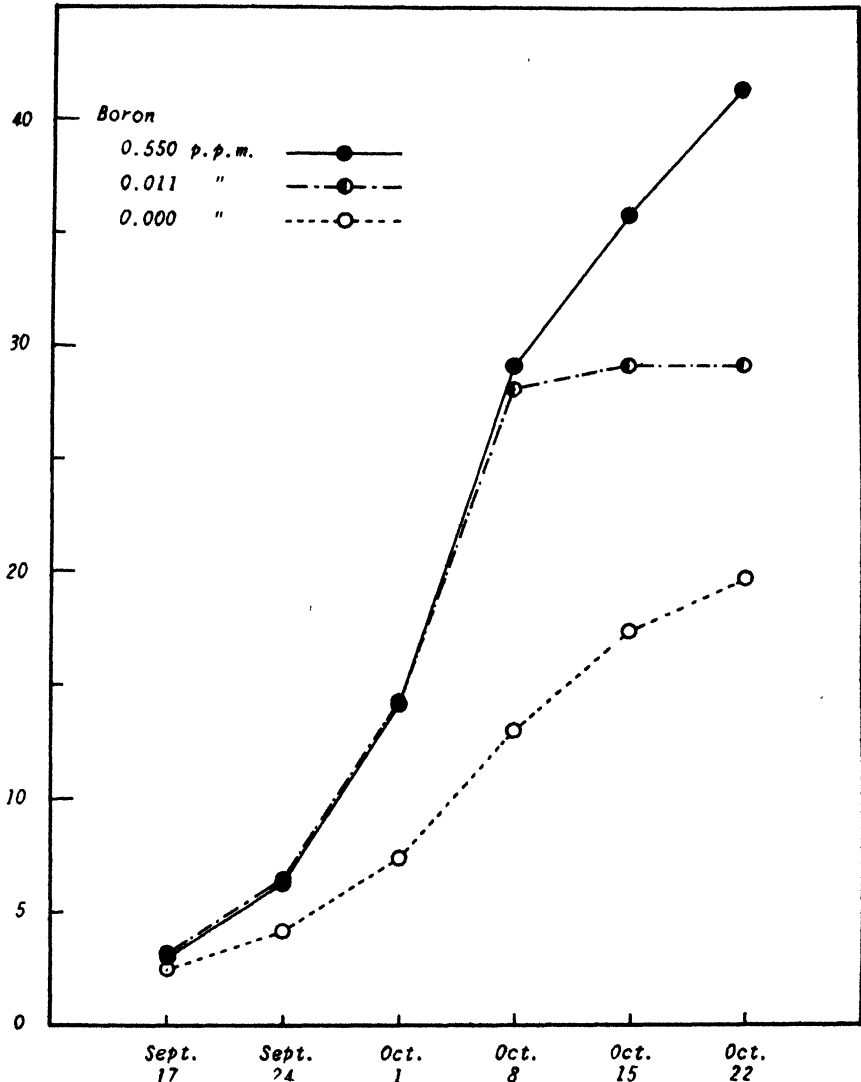


FIG. 7. Average height (cm.) of tomato plants grown in nutrient solutions deficient in boron and in solutions to which 0.011 and 0.55 ppm. boron had been added.

experiment was performed at Maryland with the Marglobe tomato. The plants were grown from November 1 to December 13 without changing the nutrient solutions. They were then grown for four more weeks after the proper changes had been made. The data of this experiment are presented in detail in table XII. At the end of 6 weeks distilled water was added to cultures whose solutions were not changed. All culture jars were of the two quart size with one plant per culture.

TABLE XII

DATA SHOWING AVERAGE HEIGHT (CM.) AND FINAL GREEN AND DRY WEIGHTS (GM.) OF TOMATO PLANTS GROWN IN NUTRIENT SOLUTIONS TO WHICH BORON HAD AND HAD NOT BEEN ADDED AT THE BEGINNING OF THE EXPERIMENT AND AFTER 6 WEEKS. AMOUNT OF BORON ADDED IS SHOWN IN PARENTHESIS AS PPM.

PERIOD ENDING 1927	(0.55)		(0.55)		(0.0)		(0.0)	
	cm.		cm.		cm.		cm.	
November 8	2.5		2.4		2.1		2.4	
15	4.7		4.4		4.4		4.6	
22	7.5		7.0		7.3		7.5	
29	13.6		12.4		13.2		13.4	
December 6	17.8		16.1		17.5		17.9	
13	24.9		22.9		20.3		21.0	
Solutions renewed with	(0.0)	(0.55)	No renewal	(0.0)	(0.55)	No renewal		
	cm.	cm.	cm.	cm.	cm.	cm.		
December 20	29.8	30.7	28.8	20.7	22.4	21.3		
26	33.2	35.1	31.7	21.9	24.3	21.3		
1928								
January 3	38.8	42.8	35.6	21.6	28.6	21.3		
10	41.8	50.0	39.8	21.8	31.8	21.2		
Number of plants in group	5	5	10	5	5	10		
Green weight	gm.	gm.	gm.	gm.	gm.	gm.		
Tops	63	66	52	34	49	22		
Roots	13	15	18	4	13	5		
Total	76	81	70	38	62	27		
Dry weight								
Tops	6.9	7.6	5.8	3.6	5.0	3.1		
Roots	1.3	1.8	1.5	0.3	1.0	0.3		
Total	8.2	9.4	7.3	3.9	6.0	3.4		

The upper part of table XII shows the average height per plant in each group on the dates indicated in the first column. For the first six weeks

little variation can be seen between the plants of the first two groups each receiving 0.55 ppm. boron. Likewise little difference exists in the height of the plants of the two groups receiving no boron for this same time period. The plants of the latter two groups are slightly shorter on December 13 than those of the first two groups, but no significant differences can be detected on December 6, one week earlier. On December 13, the first and third groups were divided, thus making six groups. The treatments are indicated in the line between the dates December 13 and 20 of the table. As shown in the observation of January 10, four weeks after these changes were made, the effect of boron on stem elongation is very interesting. The average height of plants of group one, receiving no boron, is approximately 8 cm. less than their controls. The original second group in which no solutions were renewed but which originally contained 0.55 ppm. boron has practically the same stem height value as the first half of group one. It thus appears as though a deficiency of boron were the limiting growth factor rather than a deficiency of any other element originally contained in the solution since all the elements of solutions in group one were renewed on



FIG. 8. Photograph showing tomato plants grown for six weeks in given concentrations of boron, then changed to other concentrations for a period of four weeks. The following treatments were used (reading from left to right): No boron and no renewal of solution; no boron, but solution renewed; no boron, then solution renewed plus 0.55 ppm. boron.

December 13 and only distilled water added to cultures of the original second group. The green and dry weight data shown in the lower half of the table bring out this point but to a less extent. Turning now to the second part of the table, the groups originally receiving no boron, it will be noted that after six weeks of growth without boron the plants receiving boron for the last four weeks started to grow. By January 10, four weeks later, the plants in the second half of group four were 10 cm. taller than their controls. Both green and dry weight also indicate a remarkable



FIG. 9. Photograph showing new leaf growth in tomato plants after they had been defoliated. All solutions were similar with the exception of their boron content.

Reading from left to right: No boron; 0.011 ppm.; 0.55 ppm.

increase in growth due to the action of the boron added to the nutrient solution.

This renewal of growth when the plants have not been deprived of boron for too long a period is shown in figure 8. The plants here shown are representative ones taken from groups receiving the last three treatments indicated in table XII. This remarkable influence exerted by boron on growth, especially on the formation of new cells in the growing points was observed in connection with the experiment from which data on the pectic materials

were obtained. After the leaves had been removed for analysis the defoliated plants were left standing in their respective culture media for four weeks. After this period it was observed that no new shoots had appeared on the plants of the boron-deficient solutions while new shoots and leaves were abundant on the plants in the group treated with 0.55 ppm. boron. The intermediate group of plants, those receiving 0.011 ppm. boron, contained a few new shoots and leaves. Figure 9 illustrates the appearance of representative plants from each of these three groups.

Discussion

The evidence presented by plant physiologists in recent years is such that boron must be regarded an essential element for the growth and normal development of many of the higher plants. As yet the exact function of this element is not known. To speak of it as stimulating in small concentrations and as toxic in larger concentrations means little as to its fundamental physiological relation to the plant. Toxicity is a matter of relative concentrations in the presence or absence of other substances. Even the "old" nutrient elements are toxic under certain conditions. The authors are in agreement with SOMMER AND LIPMAN (14) that to speak of the catalytic effect of an element merely expresses in other language ignorance in respect to its actual function.

BRENCHLEY (2) states, "The old 'nutrients' had certain definite characters in common, in that they were essential to plant growth, the growth being in a great degree proportional to the supply, a relatively large amount of the nutrients being not only tolerated but necessary. . . . Even those that cause increased growth or that may be essential for nutrition (boron) are not required in such quantities as potassium, phosphorus, nitrogen, etc., while there is no evidence that growth is proportional to supply." It is true that boron cannot be used in quantities nearly as great as potassium, phosphorus or nitrogen, yet in these experiments with the tomato there is evidence of a quantitative relationship between growth and the amount of boron supplied. Reference to table XI and to figures 5, 6 and 7 clearly show a difference in growth where 0.011 and 0.55 ppm. boron were used.

SOMMER (13) found in her work with monocotyledonous plants such as corn, abnormal tillering as well as withering of the growing points of the tops. This interesting growth formation is no doubt related in some way to the peculiar growth forms (see figure 4) produced in the tomato after the growing points of the stems died. Apical dominance enters the problem at this point. As soon as the influence of the terminal shoot on the development of lateral buds is removed, these lateral buds begin their growth. Such dominating influence can be eliminated by removing the terminal

growing point; it amounted to the same thing in the tomato when the terminal shoot died from a cause attributed to boron deficiency. On *a priori* reasoning the abnormal tillering of corn in SOMMER's experiments may in part be explained as a pruning effect; at least there is an interesting similarity in the behavior of these two widely different plants.

Although extremely small quantities of boron are essential to the tomato plant this element must be supplied constantly. There is apparently no reserve built up in the plant for future growth since plants grown for six weeks in solutions containing boron and then grown in boron-deficient solutions soon showed characteristic symptoms of boron-deficient injuries. This is apparent from the data given in table XII and is in agreement with WARINGTON's (17) statement, "The fact that boron can be detected in the stem, leaves, and pods of the broad bean implies that the element becomes distributed throughout the plant after absorption; and further, the need for the supply of boron to be maintained during the life of the plant indicates that the initial reserve of the element in the seed is insufficient for the needs of the plant, and that it is in some way fixed and not in a state of circulation."

A vital relationship exists between boron and the conducting tissues of the tomato. If boron is essential for cell division as seems to be the case in the meristematic tissue of growing points, its presence is just as much needed in the cambium cells where phloem and xylem tissues are forming. These are the tissues which together with the tip of the stem show greatest injury from a deficiency of boron. Both the chemical analyses and macroscopic observations indicated a failure on the part of the boron-deficient plants to remove sugar from their leaves. This is apparently related to the broken down condition of the conducting tissues. Microscopic examinations of the petioles and stems of boron-deficient plants showed phloem necrosis. These general observations are in agreement with the anatomical studies of WARINGTON (17) on *Vicia faba* grown in boron-deficient solutions.

At first it was thought the characteristic brittleness of stems and petioles of boron-deficient plants was in some way associated with pectic materials. The experimental data, however, fail to substantiate such a theory. The observation by BRECHLEY (2) that boron occurs most abundantly in bark and lignified parts, suggests that brittleness may be related to a lack of proper lignification. No conclusions upon this point appear possible from the lignin-suberin values which we have obtained.

Conclusions

The conclusions drawn from a series of water culture experiments carried out at the Universities of California and Maryland with two varieties

of tomato, Santa Clara Canner and Marglobe, may be summarized as follows:

1. The element boron in a concentration of approximately 0.5 ppm. was found necessary for the normal growth and development of the tomato plants studied.
2. Tomato plants grown in boron-deficient solutions show four distinct types of injury; (a) death of the terminal growing point of stem; (b) breaking down of the conducting tissues in the stem; (c) a characteristic brittleness of stem and petiole; and (d) roots of extremely poor growth and of a brownish unhealthy color.
3. As a result of broken down conducting tissues the boron deficient plants differed markedly from normal plants in their chemical composition. Total sugars and starch were more abundant in the leaves and stems of the boron-deficient plants while a greater amount of benzene-soluble matter was found in the leaves of the normal plants and in the stems of the boron-deficient plants.
4. Evidence is presented which shows the possibility of a quantitative relationship existing between the amount of growth and the amount of boron present in the nutrient media.
5. A concentration of 5.5 ppm. boron in the nutrient solution was toxic to the tomato plants. Symptoms of boron toxicity are quite different from deficiency injuries.

The writers wish to acknowledge their indebtedness to Director H. J. PATTERSON of the University of Maryland Agricultural Experiment Station, to the Division of Plant Nutrition, University of California and to a grant from the American Potash and Chemical Company for funds which made it possible to carry on these cooperative experiments. Special acknowledgment is due to Professor HOAGLAND for his valuable advice and criticism throughout the investigation.

THE UNIVERSITY OF MARYLAND,
THE UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. APPLEMAN, C. O., and CONRAD, C. M. The pectic constituents of tomatoes and their relation to the canned product. Univ. of Maryland Agr. Exp. Sta. Bull. 291. 1927.
2. BRENCHLEY, WINIFRED E. Inorganic plant poisons and stimulants. 2nd ed. Cambridge University Press. 1927.
3. —————, and WARINGTON, K. The rôle of boron in the growth of plants. Ann. Bot. 41: 167–187. 1927.
4. CONRAD, C. M. A biochemical study of the insoluble pectic substances in vegetables. Amer. Jour. Bot. 13: 531–547. 1926.

5. DORE, W. H. The composition of pectin: A preliminary report on the determination of galacturonic acid in pectin. Jour. Amer. Chem. Soc. **48**: 232-236. 1926.
6. JOHNSTON, EARL S. A method of studying the absorption-transpiration ratio in nutrient media. Science N. S. **52**: 517-518. 1920.
7. ————. The seasonal march of the climatic conditions of a greenhouse, as related to plant growth. Univ. of Maryland Agr. Exp. Sta. Bull. 245. 1921.
8. ————, and DORE, W. H. The relation of boron to the growth of the tomato plant. Science N. S. **67**: 324-325. 1928.
9. LESLEY, J. W. and ROSA, J. T. The improvement of tomatoes by selection. Hilgardia **2**: 25-45. 1926.
10. LIVINGSTON, B. E. Incipient drying and temporary and permanent wilting of plants, as related to external and internal conditions. Johns Hopkins Univ. Cir. March, 1917. Pp. 176-182.
11. ————, and BROWN, W. H. Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. **53**: 309-330. 1912.
12. RENNER, O. Experimentelle Beiträge zur Kenntnis der Wasserbewegung. Flora **103**: 171-247. 1911. *Idem*. Versuche zur Mechanik der Wasserversorgung. I. Der Druck in den Leitungsbahnen von Freilandpflanzen. Ber. d. bot. Ges. **30**: 576-580. 1912.
13. SOMMER, A. L. The search for elements essential in only small amounts for plant growth. Science N. S. **66**: 482-484. 1927.
14. ————, and LIPMAN, C. B. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. **1**: 231-249. 1926.
15. SWANBACK, T. R. The effect of boric acid on the growth of tobacco plants in nutrient solutions. Plant Physiol. **2**: 475-486. 1927.
16. WARINGTON, K. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. **37**: 629-672. 1923.
17. ————. The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. **40**: 27-42. 1926.

RELATION BETWEEN TOP AND ROOT SIZE IN HERBACEOUS PLANTS*

JOHN W. CRIST AND G. J. STOUT

In his Treatise on the Physiology of Plants SORAUER affirms that the plant must not be looked upon as an unchangeable organism, restricted to a definite form, but as being plastic, capable of further modification in any of its parts; its usual shape being susceptible to alteration, within certain limits, as if it were made of wax. This principle has been substantiated by the practical gardener, who, besides seeking to grow herbaceous plants in situations to which they are the best adapted by nature, often endeavors to cultivate them successfully in special localities, and, in all localities, seeks to render them more useful to his purposes.

Perhaps chief among the attempts of the gardener at altering normal physiological functions and interfering with the ordinary course of development is that of remodelling certain plants with respect to the relative size of top and root. His success in this effort has been remarkable; so much so that there might seem to be an attainable "ideal balance" between size of top and root for every particular desire or necessity. However, the limits mentioned by SORAUER must exist, making it impossible to proceed beyond a certain point with a given species, and also rendering it likely that even within the limits set by nature, increasing or decreasing the normal development of either top or root in order to favor the other part may result in danger to the plant as a whole except this be attempted by persons who thoroughly understand the plant and its conditions of life and vigor. Obviously, the aerial and subterranean portions of a higher land plant are mutually dependent, since the roots furnish the raw nutrients and water obtained from the soil and often serve as places of storage, while the tops supply the elaborated organic compounds essential for growth. It is of practical significance and also of considerable scientific interest to determine the nature of this relationship, or, more specifically, to determine how much variation in this state of reciprocity occurs naturally, and how much may be induced experimentally.

Literature

Interest in the proportionate size of the top and root of the plant is of long standing. The following quotation is from the ancient writings of VARRO (7): "In autumn and winter the roots develop more than does the leaf of the plant because they are nourished by the warmth of the roof of

* Published as paper no. 6 (n. s.) from Agricultural Experiment Station of Michigan State College.

earth, while the leaf above is cut down by the frosty air. We can learn this by observation of the wild vegetation which grows without the intervention of man; for the roots grow more rapidly than that which springs from them, but only so far as they are actuated by the rays of the sun. There are two causes of the growth of roots, the vitality of the root itself by which nature drives it forward and the quality of the soil which yields a passage more easily in some conditions than in others."

These early observations have been followed in more recent times by numerous experimental studies on the relation of top and root size, use having been made of many different species and varieties of herbaceous plants. A chronological review of the literature shows by way of generalization that there has been an increasing tendency to seek the causes underlying variations in relative growth of top and root, and also to subject the data obtained to more complete mathematical analysis and interpretation. These trends at their highest present points are exemplified in reports such as those made by WEAVER (9), WEAVER and his associates (8), TURNER (6) and PEARSALL (3, 4), the latter having concluded that the relative sizes of plant organs conform to the equation $X = CY^k$, where X and Y are the sizes of the organs, C is a constant expressing their relative sizes, and k indicates the ratio of their relative logarithmic growth rates and the spatial arrangements of their meristems. Obviously, this formula sets forth the relationship that the logarithm of stem weight is directly proportional to the logarithm of root weight. When the two time-series of logarithms are plotted against each other the result is a straight line.

In general, the available experimental evidence leads to the conclusion that herbaceous plants exhibit specific and varietal differences with respect to relative development of top and root under uniform environmental conditions. However, few if any of the many types are non-plastic, that is, do not react to environmental changes by way of showing variations in the relative sizes of aerial and underground parts. The more intensive studies, particularly those made on growth rates, indicate that though variation occurs there is a persistent tendency towards positive correlation. The present writers have not examined growth rates but have obtained final weights on the tops and roots of lettuce, radish and tomato plants from a number of different experiments and have treated the data as shown later.

Methods of procedure

EXPERIMENTAL

In some of the experiments to be reported the plants were grown in greenhouse flats; in others in flower pots of different sizes. Whether grown in flats or pots the containers were set on boards to prevent the exit of the

roots. Where pots were used, these were set on the bottom of the greenhouse bench and buried to their rims in moist sand. Any objection to the confinement of the roots in such containers can be met by the statement that this was simply one of the conditions under which top-root relations were measured. If the ultimate sizes of top and root are definitely and inevitably correlated, then the type of container might alter the degree of correlation but not destroy it nor change its character qualitatively. Furthermore, the use of flats and pots made it possible to recover the root systems readily and *in toto*. All the experiments were conducted in the greenhouse and every precaution taken to maintain uniform conditions of environment while any one of them was under way.

When the plants were grown in pots, the roots were obtained by freeing the whole mass from the pot, immersing it in a large volume of water, and gently kneading it with the hands until practically all of the soil was washed out. Following this, the root system was washed twice more in fresh water and then placed between layers of newspapers to dry off for a given length of time before the weight was taken.

When flats were used as containers, six plants were grown in each flat. The process for securing the separate root systems was that of dividing the mass of soil into six equal blocks; removing each one of the blocks by placing a square of galvanized iron under it, and immersing the block in water. From this point onward the method was the same as described previously for the plants grown in pots.

MATHEMATICAL

The top-root ratios were calculated in three ways: (1) as the quotient of mean (arithmetic) top to mean (arithmetic) root; (2) as the arithmetic mean of the individual top-root ratios, and (3) as the geometric mean of the individual ratios. The results from the use of these three methods of calculation on the same data differ sufficiently to necessitate making a choice among them. The properties of the geometric mean, when dealing with ratios, are such as to commend its use in preference to the others. On this mean and its probable error see YULE (10), KELLEY (2), and CRIST (1).

When the number of individuals in any lot of plants was less than 25, PEARSON'S (5) factors for the correction of the standard deviation were used and the corrected standard deviation substituted in all other calculations where the employment of the standard deviation was required.

The product-moment formula ($r = \frac{P}{\sigma_x \cdot \sigma_y}$) was used in calculating the coefficients of correlation. The probable error of r was obtained with the formula: $P.E._r = \frac{1 - r^2}{\sqrt{N}}$. 0.6745.

No difference less than three times its probable error was considered significant, the probable error of the difference having been determined through the formula: $P.E._d = 0.6745 \sqrt{\sigma_1^2 + \sigma_2^2}$.

Interpretation of the significance of the coefficient of correlation was made according to the following rules: (a) If r is 0.5 (plus or minus) or greater, correlation is practically certain. (b) In order to be reliable r must be at least four times its probable error. (c) If, under various conditions of experimentation, r persists in having the same sign, though not always significant in itself, there is some evidence of a general though weak relationship of the character of this sign between the two variables.

EXPERIMENTS WITH LETTUCE (GRAND RAPIDS FORCING VARIETY)

USE OF RAW SULPHUR AND SUPERPHOSPHATE (C.P. AND COMMERCIAL).—The seed was sown broadcast in greenhouse flats, the seedlings selected at the proper time, and transplanted to other flats (6 per flat) in which they were grown for a period of 51 days. The soil was a fertile fine sandy loam. The data appear in table I.

In each case shown in table I the ratio of the average top to average root R_1 is less than R (the arithmetic mean of the ratios for the plants individually), while Mg lies between these two. The probable error for any Mg is higher than for the corresponding R . This is the relationship between the three forms of average which holds as a general rule. Accordingly, only the geometric mean will be given and used hereafter in making comparisons.

The number of plants per lot was small, by design. This necessitated large differences, if they were to be significant. Under these conditions, superphosphate when applied either alone or along with raw sulphur, except in lot 4 where 800 pounds of the sulphur per acre were used, significantly increased the top-root ratio as compared with lots 1 and 2 where sulphur only was applied.

The ratios do not vary directly with the total weights of average plants. The smallest plants have the highest ratio, the largest plants have the second largest ratio, while the plants intermediate in size have the lowest ratios.

With so few plants none of the coefficients of correlation, including that for all the plants taken together, is significant. However, it is noteworthy that all of them are positive in character, one (lot 5) being above 0.5, and also that they do not vary consistently in magnitude with either the top-root ratios or with the average total weights of the plants.

INFLUENCE OF CALCIUM NITRATE, POTASSIUM CHLORIDE AND SUPERPHOSPHATE.—The plants were grown in a very poor medium sandy loam soil in six inch pots. The nutrient treatments were applied in solution form at the beginning of the experiment. Further details and other data for this

experiment may be found in Michigan Experiment Station Tech. Bull. 74. 1926. See table II for the results.

TABLE I
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

Lot	NUMBER OF PLANTS	SOIL TREATMENT	AVERAGE GREEN WEIGHT		R_t^a	R^a	Mg^a	r^a
			Top	Root				
		<i>lbs. per acre</i>	<i>gm.</i>	<i>gm.</i>				
1	6	Sulphur, 400	86.8 ± 3.63	11.5 ± 0.31	7.52	7.56 ± 0.265	7.48 ± 0.270	0.433 ± 0.224
2	6	Sulphur, 800	89.3 ± 1.07	12.2 ± 1.07	7.33	7.54 ± 0.389	7.44 ± 0.401	0.169 ± 0.267
3	6	c.p. $CaH_4(PO_4)_2$, 1200	93.5 ± 3.43	10.0 ± 0.06	9.35	9.57 ± 0.470	9.46 ± 0.479	0.367 ± 0.238
4	6	c.p. $CaH_4(PO_4)_2$, 1200						
		plus sulphur, 800	90.6 ± 4.00	10.8 ± 0.02	8.39	8.38 ± 0.330	8.32 ± 0.337	0.333 ± 0.245
5	6	Commercial $CaH_4(PO_4)_2$, 1200	99.2 ± 2.52	9.3 ± 0.06	10.60	10.94 ± 0.550	10.80 ± 0.565	0.578 ± 0.186
6	6	Commercial $CaH_4(PO_4)_2$, 1200						
		plus sulphur, 400	84.0 ± 2.51	7.3 ± 0.07	11.50	12.53 ± 1.170	12.02 ± 1.255	0.296 ± 0.251
All	36							0.319 ± 0.100

^a In all tables " R_t " is ratio of average top to average root; " R ", average ratio of individual plants; " Mg ", geometric mean of individual ratios; " r ", coefficient of correlation.

TABLE II
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
			TOP	ROOT	WHOLE PLANT		
			gm.	gm.	gm.		
1	14	Check	7.4 ± 0.27	7.9 ± 0.22	15.3	0.93 ± 0.029	0.481 ± 0.139
2	7	Ca(NO ₃) ₂ , 100	14.6 ± 0.83	11.7 ± 0.74	26.3	1.25 ± 0.023	0.761 ± 0.107
3	7	Ca(NO ₃) ₂ , 300	14.5 ± 0.84	9.1 ± 0.39	23.6	1.58 ± 0.102	0.276 ± 0.235
4	7	Ca(NO ₃) ₂ , 600	20.4 ± 1.19	12.3 ± 0.53	32.7	1.64 ± 0.103	0.398 ± 0.214
5	7	Ca(NO ₃) ₂ , 600 plus KCl, 300	22.9 ± 1.29	13.2 ± 0.25	36.1	1.71 ± 0.077	0.591 ± 0.166
6	7	Ca(NO ₃) ₂ , 600 plus CaH ₂ (PO ₄) ₂ , 300	35.0 ± 1.75	18.1 ± 1.06	53.1	1.95 ± 0.077	0.621 ± 0.156
7	7	Ca(NO ₃) ₂ , 600 plus KCl, 300 plus CaH ₂ (PO ₄) ₂ , 300	33.9 ± 2.16	16.6 ± 0.91	50.5	2.03 ± 0.166	0.243 ± 0.239
All	56						0.879 ± 0.020

Again, as in the preceding experiment, the number of plants per lot, except lot 1, was small. Nevertheless, table II shows that any nutrient treatment increased the top-root ratio and the ratios show an unmistakable tendency to be continuously higher as more complete fertilization of the soil obtains. The average weight of plant increases along with the ratios except in lots 3 and 7. In these cases the increased ratio does not indicate an absolute weight of top greater than in the preceding lot but is brought about by a reduction in the root which exceeds that in the top, thus leaving the top actually smaller, but still relatively larger than the root.

Though but one of the coefficients of correlation (lot 2) is significant, all are positive in sign; three of them (lots 2, 5, 6) being greater than 0.5. Here, as in the former experiment, these coefficients sustain no uniform relation to either the top-root ratios or the average total weights of the plants. When thrown together into one group, the 56 plants show strong positive correlation, the coefficient being 0.879 ± 0.020 .

USE OF ACID AND NEUTRAL SOIL WITH APPLICATIONS OF HYDRATED LIME AND SUPERPHOSPHATE.—Complete details of this experiment, which consisted primarily in a study of the acid tolerance of lettuce, can be found in Michigan State College Experiment Station Tech. Bull. 71. 1926, pages 14–18. The plants were grown in flats. Two types of soil were used. One soil was a mixture of two-thirds muck and one-third sand with a lime requirement of 20,000 pounds $\text{Ca}(\text{OH})_2$ (JONES method). The second soil was a neutral fine sandy loam. The data, presented in table III, were taken when the plants had reached vegetative maturity. The number of plants per lot was larger than in either of the two former experiments.

The twenty-seven lots of plants listed in table III fall into five groups, namely, lots 1 to 6 with increasing amounts of lime; lots 7 to 10 with increasing applications of both lime and superphosphate; lots 12 to 17 with superphosphate constant while the lime treatments increase in size; lots 19 to 22 on neutral loam soil with superphosphate constant and lime applications increasing in size; lots 23 to 27 on neutral loam soil with increasing quantities of lime. Within any one of the five groups, the top-root ratios are approximately equal for the several lots, while at the same time, the average size of whole plants decreases with increased size of application of the materials used for soil treatment, due to both top and root being progressively smaller to about the same degree.

The effect of soil type is very pronounced. The top-root ratios on the acid muck-and-sand mixture are conspicuously higher than on the neutral sandy loam. This holds true where comparable treatments were used as well as for the checks. Relative root development in the acid muck-and-sand soil mixture was greatly reduced. This went to a very great extreme

TABLE III
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	SOIL TREATMENT ^a	AVERAGE GREEN WEIGHT			Mg	r
			TOP	ROOT	WHOLE PLANT		
			gm.	gm.	gm.		
1 ^a	24	Check	65.7 ± 2.24	8.8 ± 0.34	74.5	7.58 ± 0.254	0.540 ± 0.100
2	12	Ca(OH) ₂	70.3 ± 2.65	10.2 ± 0.47	80.5	6.94 ± 0.365	0.314 ± 0.176
3	12	Ca(OH) ₂	58.7 ± 1.93	9.7 ± 0.36	68.4	6.08 ± 0.368	-0.265 ± 0.181
4	12	Ca(OH) ₂	38.2 ± 1.45	6.0 ± 0.24	44.2	6.40 ± 0.237	0.408 ± 0.163
5	12	Ca(OH) ₂	26.0 ± 1.24	3.2 ± 0.19	29.2	8.32 ± 0.555	0.443 ± 0.156
6	12	Ca(OH) ₂	15.4 ± 0.94	1.8 ± 0.16	17.2	9.10 ± 0.742	0.678 ± 0.105
7	12	Ca(OH) ₂	69.4 ± 3.03	5.4 ± 0.22	72.7	12.96 ± 1.821	0.358 ± 0.170
8	12	Ca(OH) ₂	70.7 ± 2.03	6.4 ± 0.32	77.1	11.36 ± 0.567	0.447 ± 0.156
9	12	Ca(OH) ₂	60.8 ± 1.94	4.9 ± 0.34	65.7	12.89 ± 1.008	0.287 ± 0.179
10	12	Ca(OH) ₂	56.0 ± 2.11	4.3 ± 0.29	60.3	13.41 ± 0.613	0.618 ± 0.121
11	18	c.p. KH ₂ PO ₄	21.2 ± 1.71	3.2 ± 0.19	24.4	6.27 ± 0.414	0.664 ± 0.089
12	12	CaH ₂ (PO ₄) ₂	24.7 ± 1.42	4.2 ± 0.28	28.9	5.95 ± 0.350	0.608 ± 0.123
13	12	Ca(OH) ₂	81.8 ± 2.08	11.3 ± 0.46	93.1	7.33 ± 0.193	0.712 ± 0.096
14	12	Ca(OH) ₂	75.3 ± 2.04	7.7 ± 0.31	83.0	9.94 ± 0.666	-0.460 ± 0.154
15	12	Ca(OH) ₂	65.9 ± 1.30	10.6 ± 0.59	76.5	6.39 ± 0.309	0.522 ± 0.142
16	12	Ca(OH) ₂	60.3 ± 2.23	8.8 ± 0.41	69.1	6.89 ± 0.361	0.356 ± 0.170
17	12	Ca(OH) ₂	72.1 ± 2.58	11.1 ± 0.45	83.2	6.52 ± 0.411	-0.075 ± 0.194
18 ^b	12	CaH ₂ (PO ₄) ₂	16.9 ± 0.85	7.3 ± 0.36	24.2	2.30 ± 0.082	0.645 ± 0.114
19	12	Ca(OH) ₂	18.1 ± 0.84	7.8 ± 0.27	25.9	2.28 ± 0.199	0.729 ± 0.091
20	12	Ca(OH) ₂	21.5 ± 1.36	6.7 ± 0.25	28.2	3.15 ± 0.162	0.622 ± 0.119
21	12	Ca(OH) ₂	17.9 ± 0.94	6.7 ± 0.36	24.6	2.65 ± 0.125	0.407 ± 0.163
22	12	Ca(OH) ₂	14.0 ± 0.82	4.7 ± 0.35	18.7	3.12 ± 0.356	0.508 ± 0.144
23	12	Check	17.9 ± 0.83	8.0 ± 0.40	25.9	2.24 ± 0.090	0.605 ± 0.123
24	12	Ca(OH) ₂	18.7 ± 1.15	7.5 ± 0.28	26.2	2.42 ± 0.120	0.589 ± 0.127
25	12	Ca(OH) ₂	14.3 ± 0.93	5.6 ± 0.44	19.9	2.62 ± 0.121	0.748 ± 0.086
26	12	Ca(OH) ₂	9.6 ± 0.68	3.1 ± 0.11	12.7	2.99 ± 0.196	0.439 ± 0.157
27	12	Ca(OH) ₂	7.6 ± 0.48	2.6 ± 0.17	10.2	3.02 ± 0.098	0.797 ± 0.071
All	342						0.543 ± 0.026

^a Lots 1 to 17 inclusive, soil was muck-and-sand mixture.

^b Lots 18 to 27 inclusive, soil was neutral sandy loam.

^c All nutrient substances of commercial grade, unless noted otherwise.

in lots 7 to 10 where the lime and the superphosphate were increased together.

In regard to the twenty-seven coefficients of correlation three, none of which is at all significant, are negative. Twenty-four of the coefficients, ten of which are above 0.5 and significant, are positive. Variation in the size of the coefficients is not consistent to any important extent with the soil treatments or the changes in the size of the plants and the ratios produced by these treatments. Correlation with all the plants taken in a single group is positive and clearly significant (0.543 ± 0.026).

INFLUENCE OF LENGTH OF DAY AS MODIFIED BY NUTRIENT TREATMENT.—The plants were grown singly, to vegetative maturity, in six-inch pots, the soil being a fine sand, low in fertility. Various nutrients were applied in different ways. The extra illumination of the plants designated as long-day plants was accomplished by means of 1000 watt Mazda lamps, socketed in porcelain enameled steel dome reflectors suspended at a height of 4.5 feet above the bench. Three of these bulbs, evenly spaced, were used over a bench 21 feet long and 5 feet wide, the pots containing the plants being distributed so as to give even illumination and also being systematically shifted about in their places once each week. The experiment was conducted during the short cloudy days of the winter season. The day for the long-day plants was lengthened 6 hours as an average. The plants of the short-day lots were covered each day at 3:00 P. M. and left covered until 9 o'clock the following morning. The covers consisted of specially built light-tight boxes each 10 feet long, 5 feet wide and 3 feet high, with light-tight ventilators arranged in each end. See table IV for the results.

An examination of table IV makes it clear that the top-root ratios were increased as the period of illumination was shortened. The differences for lots 1, 6 and 7, lots 2 and 8, and lots 3 and 9 are large and certainly significant. In the long-day group the nutrient treatments elevated the ratios decidedly. The increases in the short-day group are only apparent. Within groups, the increased ratios are associated with increased size of plant and greater actual weight of top, though the three largest ratios (lots 7, 8, 9) obtain with plants among the smallest in size. While the nitrate treatments of the short-day plants elevated the top-root ratios apparently, large plants were not produced as with the long-day treatments. Though raw materials from the soil have been sufficient, assimilable food products were not ample to give greater total growth more evenly distributed between top and root.

All of the coefficients of correlation are positive, three of them (lots 1, 6 and 9) giving evidence of certainty. The coefficient (0.685 ± 0.026) for all the plants, thrown together, is such as to make positive correlation certain.

TABLE IV
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

LOT	NUMBER OF PLANTS	DAY	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			<i>gm. per pot</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
1	21	Long	Check	11.2 ± 0.32	14.5 ± 0.65	25.7	0.79 ± 0.023	0.700 ± 0.075
2	21	"	Ca(NO ₃) ₂ , 1	49.8 ± 1.58	18.9 ± 0.84	68.7	2.71 ± 0.177	0.355 ± 0.129
3	21	"	*Ca(NO ₃) ₂ , 1	54.2 ± 2.28	11.3 ± 0.62	65.5	4.90 ± 0.373	0.363 ± 0.128
4	21	"	Ca(NO ₃) ₂ , 1 plus CaH ₄ (PO ₄) ₂ , 1	58.8 ± 1.58	15.1 ± 0.47	73.9	3.94 ± 0.171	0.133 ± 0.144
5	21	"	*Ca(NO ₃) ₂ , 1 plus *CaH ₄ (PO ₄) ₂ , 1	75.2 ± 1.77	16.7 ± 0.84	91.9	4.72 ± 0.320	0.385 ± 0.125
6	15	Normal	Check	11.0 ± 0.62	4.5 ± 0.47	15.5	2.84 ± 0.590	0.713 ± 0.086
7	20	Short	Check	8.5 ± 0.32	1.4 ± 0.13	9.9	6.88 ± 1.101	0.417 ± 0.125
8	22	"	Ca(NO ₃) ₂ , 1	15.1 ± 0.52	1.5 ± 0.11	16.6	10.84 ± 1.000	0.352 ± 0.126
9	22	"	*Ca(NO ₃) ₂ , 1	13.4 ± 0.59	1.7 ± 0.14	15.1	8.74 ± 0.701	0.582 ± 0.095
All	184							0.685 ± 0.026

* Applied cumulatively in fourth portions.

INFLUENCE OF LENGTH OF DAY IN CONJUNCTION WITH NUTRIENT TREATMENTS (SECOND EXPERIMENT).—This experiment differed from the preceding one in that it was performed during the spring season (April 17 to May 23), the former having been conducted in midwinter. The short-day plants had a day of eight hours, the long-day plants 16 to 18 hours, while the normal day was approximately 10 to 12 hours in length. Otherwise, the two experiments were duplicated as to methods. The results are shown in table V.

The data in table V confirm the main conclusion that was evident from the results of the preceding experiment, namely, that the top-root ratio was raised as the day was shortened, either with or without the nitrate treatments. In two of the three groups the nitrate of itself significantly increased the ratio. Again, the highest ratios are for the short-day plants and are associated with the smallest plants which, at the same time, have the lowest actual weight of top, as well as root.

All of the coefficients of correlation are positive, three of them being about 0.5 and no one of these three less than five times its probable error. The coefficient for the 178 plants is positive, more than 0.5, and is 21 times its probable error.

EXPERIMENTS WITH RADISH PLANTS (SCARLET GLOBE VARIETY)

USE OF NUTRIENT SUBSTANCES ON TWO SOILS OF DIFFERENT COMPOSITION.—One of the two soils, the one designated as A in table VI was composed of five parts fine sandy loam and one part medium coarse sand; the other, B, of these types of soil, with the proportions reversed. These soils were treated as shown in table VI, and used in six-inch pots in which the plants were grown singly.

Table VI shows that the nutrient treatments increased the average size of plant on each one of the two soils but the top-root ratio was not changed significantly. With one exception (lot 6) the average plant was larger on soil B than soil A, while at the same time the ratio of top to root is consistently lower, though the differences are not strictly real except as between lots 1 and 2.

The ten coefficients of correlation are positive; seven of them being above 0.5. Of these seven, three are fully significant. The coefficient (0.694 ± 0.031) for all the plants indicates certain positive correlation.

EFFECT OF LENGTH OF DAY AS MODIFIED BY NUTRIENT TREATMENTS

This experiment was made simultaneously with the one on lettuce, the data for which are contained in table IV, using the same general methods of procedure. The period of illumination for the long-day plants was six

TABLE V
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR LETTUCE PLANTS

Lot	Number of Plants	Day	Soil Treatment	Average Green Weight			Mg	r
				Top	Root	Whole Plant		
			<i>gm. per pot</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
1	30	Long	Check	54.5 ± 1.43	34.3 ± 0.74	88.8	1.58 ± 0.033	0.625 ± 0.076
2	30	"	NaNO ₃ , 1	79.4 ± 0.85	39.4 ± 0.72	118.8	2.03 ± 0.031	0.543 ± 0.086
3	29	Normal	Check	55.4 ± 1.21	26.3 ± 0.62	81.7	2.11 ± 0.055	0.324 ± 0.111
4	30	"	NaNO ₃ , 1	83.0 ± 1.13	39.8 ± 0.99	122.8	2.12 ± 0.053	0.340 ± 0.109
5	29	Short	Check	46.9 ± 0.88	17.8 ± 0.51	64.7	2.67 ± 0.064	0.491 ± 0.095
6	30	"	NaNO ₃ , 1	68.2 ± 1.19	17.3 ± 0.46	85.5	3.98 ± 0.108	0.295 ± 0.113
All	178							0.573 ± 0.027

TABLE VI
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR RADISH PLANTS

LOT	NUMBER OF PLANTS	SOIL	SOIL TREATMENT	AVERAGE GREEN WEIGHT			Mg	r
				TOP	ROOT	WHOLE PLANT		
			<i>gm. per pot</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
1	12	A	Check	3.3 ± 0.20	6.2 ± 0.19	9.5	0.51 ± 0.026	0.763 ± 0.082
2	15	B	Check	3.2 ± 0.16	7.8 ± 0.42	11.0	0.41 ± 0.017	0.584 ± 0.115
3	12	A	Ca(NO ₃) ₂ , 1	3.5 ± 0.19	7.0 ± 0.43	10.5	0.49 ± 0.057	0.356 ± 0.170
4	15	B	Ca(NO ₃) ₂ , 1	5.6 ± 0.33	14.3 ± 0.78	19.9	0.39 ± 0.020	0.621 ± 0.107
5	12	A	KCl, 1	3.6 ± 0.26	7.7 ± 0.38	11.3	0.45 ± 0.034	0.590 ± 0.127
6	15	B	KCl, 1	2.9 ± 0.13	8.0 ± 0.38	10.9	0.36 ± 0.020	0.566 ± 0.119
7	12	A	Ca(NO ₃) ₂ , 1 plus KCl, 1	3.9 ± 0.34	7.4 ± 0.28	11.3	0.50 ± 0.046	0.265 ± 0.181
8	15	B	Ca(NO ₃) ₂ , 1 plus KCl, 1	3.6 ± 0.17	9.5 ± 0.36	13.1	0.38 ± 0.019	0.533 ± 0.125
9	6	A	CaH ₂ (PO ₄) ₂ , 1	4.2 ± 0.24	8.0 ± 0.66	12.2	0.53 ± 0.029	0.586 ± 0.181
10	15	B	CaH ₂ (PO ₄) ₂ , 1	4.2 ± 0.25	9.1 ± 0.54	13.3	0.47 ± 0.061	0.387 ± 0.148
All	129							0.694 ± 0.031

TABLE VII
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR RADISH PLANTS

Lot	Number of Plants	Day	Soil Treatment	Average Green Weight			Mg	r
				Top	Root	Whole Plant		
			<i>gm. per pot</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
1	22	Long	Check	3.5 ± 0.09	7.0 ± 0.26	10.5	0.51 ± 0.023	0.261 ± 0.134
2	21	"	Ca(NO ₃) ₂ , 1	7.6 ± 0.46	11.8 ± 0.51	19.4	0.62 ± 0.031	0.637 ± 0.088
3	22	"	KCl, 1	3.5 ± 0.13	6.1 ± 0.39	9.6	0.61 ± 0.044	0.487 ± 0.110
4	22	"	Ca(NO ₃) ₂ , 1 plus KCl, 1	8.6 ± 0.43	12.3 ± 0.53	20.9	0.70 ± 0.059	0.212 ± 0.138
5	15	Normal	Check	2.1 ± 0.03	3.2 ± 0.19	5.3	0.67 ± 0.059	0.131 ± 0.171
6	16	Short	Check	1.6 ± 0.09	1.1 ± 0.14	2.7	1.74 ± 0.216	0.511 ± 0.125
7	16	"	Ca(NO ₃) ₂ , 1	2.2 ± 0.19	1.1 ± 0.11	3.3	2.10 ± 0.486	0.444 ± 0.136
8	14	"	KCl, 1	1.6 ± 0.11	1.2 ± 0.12	2.8	1.62 ± 0.249	0.558 ± 0.124
All	148							0.824 ± 0.018

hours in excess of the normal day, while that for the short-day plants was four to five hours less than normal. The soil was a medium coarse sand, low in fertility. Table VII gives the results.

With these plants (table VII), as with the lettuce plants (table IV), the top-root ratio increased as the day was shortened, the difference between lot 1 and lot 6 being highly significant. The increases in the ratio are accompanied by decreases in plant size, development in lots 6, 7 and 8 being very meager. Increases in the ratio due to nutrient treatments are only apparent. As to size of plant, the nitrate was effective, more effective with the long than short day.

Each of the eight coefficients of correlation is positive in character. Among the three that exceed 0.5, lot 2 is significant. The coefficient for the plants as a whole (0.824 ± 0.018) is especially high and reliable.

EXPERIMENTS WITH TOMATO PLANTS

EFFECT OF SIZE OF CONTAINER ON TOP-ROOT RELATION.—Pure line seed of the John Baer variety was sown on July 28. On August 14, the seedlings were potted singly in three-inch pots and remained in these until September 7. On the latter date, 51 of the plants were selected at random and weights of their tops and roots secured. Forty-one plants were shifted to five-inch pots, 41 to eight-inch pots, and 41 to 10-inch pots on September 9. The soil, a fine sandy loam, high in organic matter, was the same for each of the four lots. No manuring of any kind was done. The experiment was concluded on October 10, when the plants in the five-inch pots were just beginning to show symptoms of deterioration because of becoming pot-bound. The leaves and stem were weighed separately. See table VIII for the results.

The data in table VIII make it practically certain that the ratio of top to root rose as larger pots were used. This holds true whether the calculations are based on whole top, on stem, or on leaves. The plants grew to greater size in the larger containers, with both stem and leaves increasing in weight and each of these making greater relative growth than the root. The leaves constitute a greater percentage of the entire top than the stem, and gain in this respect occurs while the stem loses as the pots are larger. Furthermore, the gain in size relative to the roots is greater for the leaves than it is for the stem. The ratio of top to root for lot 2 as compared with lot 1 is not significantly different, while with lots 3 and 4 the ratios are clearly higher.

All of the coefficients of correlation are positive and all fully significant. Six of the nine are above 0.5. Though the differences are not always equal to three times their probable errors, the indication that the degree of corre-

TABLE VIII
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

Lot	Number of Plants	Size of Pot	Average Green Weight				Mg	r	
			Aerial Part*		Root	Total			
				gm.					
1	51	3	Top	4.7 ± 0.24	1.8 ± 0.09	6.5	2.19 ± 0.055	0.899 ± 0.018	
			Top	25.3 ± 1.27	12.5 ± 0.34	37.8	1.94 ± 0.060	0.718 ± 0.051	
			Stem	10.6 ± 0.40	12.5 ± 0.34	23.1	0.84 ± 0.016	0.793 ± 0.039	
			Leaves	14.7 ± 0.90	12.5 ± 0.34	27.2	1.10 ± 0.049	0.663 ± 0.059	
3	41	8	Top	57.0 ± 1.47	19.1 ± 0.35	76.1	2.96 ± 0.074	0.471 ± 0.081	
			Stem	22.7 ± 0.57	19.1 ± 0.35	41.8	1.18 ± 0.028	0.504 ± 0.078	
			Leaves	34.2 ± 0.94	19.1 ± 0.35	53.4	1.78 ± 0.043	0.462 ± 0.083	
			Top	82.9 ± 0.77	26.3 ± 0.36	109.2	3.17 ± 0.043	0.457 ± 0.083	
4	41	10	Stem	30.2 ± 0.35	26.3 ± 0.36	56.5	1.15 ± 0.049	0.500 ± 0.078	
			Leaves	52.7 ± 0.55	26.3 ± 0.36	79.0	2.01 ± 0.032	0.345 ± 0.092	
			Top						0.948 ± 0.005
			Stem						0.890 ± 0.013
All	174	All	Leaves					0.881 ± 0.013	

* Part of plant set in ratio to and correlated with root.

lation lessened as the containers were larger and that the correlation between root and stem was the most pronounced, is quite certain. This means that the degree of correlation lessened as the plants became larger and the ratios of aerial to underground parts increased. Correlation of top and root for the entire number of plants (174) is nearly perfect, while tending to be slightly less for stem and root, and for leaves and root.

INFLUENCE OF DEFLOWERING ON PRUNED AND UNPRUNED PLANTS.—In this experiment, the plants were of the Grand Rapids Forcing variety and were brought along to the stage for transplantation to pots in the same manner as those of the preceding experiment. They were transplanted to six-inch pots and grown therein on a fine sandy loam soil, well supplied with organic matter, for a period of 33 days. One lot of 84 plants was kept trained to a single stem, another lot of the same number allowed to go unpruned. Forty-two plants of each of these two groups had the flowers of the clusters (3 clusters mostly) removed as they opened. See table IX for the data.

Table IX shows some differences which appear consistent, though none of them is great enough to be considered significant. The plants are larger and the ratios higher for the unpruned plants than for the pruned and in both of these two groups larger plants with higher ratios obtain for those from which the flowers were not removed. Thus, higher ratios are apparently associated with the heavier plants.

With the eighteen coefficients of correlation, thirteen are greater than 0.5, and no one of the eighteen is rendered unreliable by the size of its probable error. Though the differences in the coefficients are consistent, being higher for the unpruned than the pruned, and higher for the plants from which the flowers were removed, they are too small, without exception, to be significant.

EFFECT OF BOTTOM HEAT ON PRUNED AND UNPRUNED PLANTS.—The work here differed from that for which the results have been given in table IX only in that bottom heat was supplied to the bench wherein the pots were placed. This elevated the temperature of the plants as a whole but especially that of the soil and the roots it contained. The arrangement for bottom heat was that of steam pipes swung under the bench at a distance of about 18 inches from the bottom of the bench, and heavy burlap, reaching to the floor, hung around the sides of the bench. Heat was kept on during the nights, sufficiently to hold the temperature of the moist sand about the pots at a point averaging about 15 degrees above that of another bench not provided with bottom heat. The results are presented in table X.

In table X, the top-root ratios are higher for the unpruned than the pruned plants, though the differences are small and insignificant. Comparing the data with those from table IX for corresponding groups of plants

TABLE IX
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

LOT	NUMBER OF PLANTS	GROUP	FLOWERS	AVERAGE GREEN WEIGHT			Mg.	r
				AERIAL PART*	Root	TOTAL		
1	42	Pruned	Off	gm. Top	gm. 9.2 ± 0.17	gm. 36.6	2.92 ± 0.067	0.573 ± 0.070
				Stem	9.2 ± 0.17	22.0	1.36 ± 0.025	0.644 ± 0.061
				Leaves	9.2 ± 0.17	23.9	1.54 ± 0.047	0.501 ± 0.078
2	42	Pruned	On	Top	10.1 ± 0.21	41.3	3.01 ± 0.105	0.510 ± 0.077
				Stem	10.1 ± 0.21	24.1	1.37 ± 0.038	0.565 ± 0.070
				Leaves	10.1 ± 0.21	27.3	1.63 ± 0.070	0.460 ± 0.082
All	84			Top				0.405 ± 0.061
				Stem				0.444 ± 0.059
				Leaves				0.362 ± 0.064
3	42	Unpruned	Off	Top	10.4 ± 0.22	44.1	3.17 ± 0.081	0.668 ± 0.057
				Stem	10.4 ± 0.22	26.3	1.51 ± 0.032	0.696 ± 0.054
				Leaves	10.4 ± 0.22	28.2	1.64 ± 0.050	0.626 ± 0.060
4	42	Unpruned	On	Top	9.8 ± 0.20	44.5	3.44 ± 0.114	0.520 ± 0.076
				Stem	9.8 ± 0.20	26.1	1.63 ± 0.046	0.549 ± 0.073
				Leaves	9.8 ± 0.20	28.3	1.80 ± 0.077	0.463 ± 0.082
All	84			Top				0.577 ± 0.049
				Stem				0.607 ± 0.047
				Leaves				0.526 ± 0.053

* Part of plant set in ratio to and correlated with root.

TABLE X
TOP-ROOT RATIOS AND COEFFICIENTS OF CORRELATION FOR TOMATO PLANTS

Lot	Number of Plants	Treatment (Bottom Heat)	Average Green Weight			Mg	r
			Aerial Part*	Root	Total		
1	47	Unpruned	Top	gm. 11.8 ± 0.16	gm. 56.5	3.70 ± 0.059	0.717 ± 0.069
			Stem	11.8 ± 0.16	30.0	1.52 ± 0.021	0.748 ± 0.043
			Leaves	11.8 ± 0.16	38.3	2.18 ± 0.042	0.687 ± 0.052
2	51	Pruned	Top	47.7 ± 1.18	13.1 ± 0.20	3.57 ± 0.067	0.675 ± 0.051
			Stem	19.0 ± 0.39	32.1	1.44 ± 0.025	0.614 ± 0.059
			Leaves	28.7 ± 0.82	41.8	2.12 ± 0.047	0.675 ± 0.051
All	98	Top Stem Leaves					0.690 ± 0.036
							0.661 ± 0.038
							0.671 ± 0.038

* Part of plant set in ratio to and correlated with root.

(lots 2 and 4) it becomes apparent that the use of bottom heat greatly increased the size of average plants. It also led to higher top-root ratios, the difference for the pruned plants being significant.

Each of the nine coefficients of correlation is positive, above 0.5 and fully reliable. None of the apparent differences between the coefficients that are comparable are significant. Better correlation of aerial parts with root obtained than in the preceding experiment, but the differences are only apparent.

Discussion and conclusions

Under any given circumstances an herbaceous plant has a definite possibility as regards total growth and ultimate size. But, while the plant is a unit, functioning and developing as an organic whole, there are distinctions of degree as regards the top and root. Significant variations in the relative distribution of growth between top and root occur naturally and may be readily induced by experimentation. The two parts are dependent, and, in general, they increase together, but not in a continuous and fixed proportion, whatever the time in the life cycle and the environment may happen to be. This is fully substantiated by comparisons of top-root ratios, even when the data are subjected to the standard tests of statistical analysis and the geometric type of average used in the process of interpretation. PEAR-SALL (4) points out that the value of k (ratio of average logarithmic growth rates) in the equation $S = eR^k$ varies with conditions of growth, as well as the type of plant. With etiolated seedlings of *Pisum sativum*, k was 2 to 3 times as great as when the plants were grown in the light. High nitrogen also elevated the value of k .

Though the relative development of top and root varies widely in nature and under experimental conditions, the two parts show a persistent interdependence on the basis of their respective final masses. While this relationship is neither markedly close nor constant in degree, it is present, in general, as a permanent characteristic. Top and root in higher plants, except in a few forms like certain species of *Tillandsia* which are rootless, other organs discharging the functions of roots, vary concomitantly. Generally speaking, correlation between top and root is positive, or in other words, increased size of top is accompanied by increased size of root or *vice versa*. This principle is deducible from the correlation coefficients that have been presented, though not as certainly from the top-root ratios. The ratios are somewhat inadequate, based as they are on the absolute values of weight. The coefficients of correlation which are derived through deviations from the line of average relationship afford the necessary criteria.

An examination of the preceding tables shows little or no tendency of the size of the plant to be dependent upon or governed by the degree of

correlation between root and top with respect to final mass. Correlation is certainly positive in character, but it does not show itself to be stronger as the plants average larger in total weight, nor less as they average smaller. In fact, if there be any relationship at all of this kind, it tends to be negative rather than positive. More often, the higher coefficients of correlation are associated with average plants that are relatively smaller. From this it may be inferred that when conditions are such as to leave the growth of the whole plant less restricted, correlation between top and root appears to be lessened.

These facts help to explain the wide range of plant adaptation observed in nature. In so far as survival, establishment and perpetuation hinge upon changes in relative development of top and root to meet existing conditions, the margin of possibility is quite great. Likewise, the position of the practical grower is rendered advantageous. He has considerable latitude by way of opportunity to alter relative size of top and root in seeking to realize his desires. An "ideal balance" from the gardener's standpoint is one which promotes the plant's thrifty development, while at the same time its growth respecting time, amount, type and place (localization in plant part) is such as to suit his material purpose. When the fact of the plant's plasticity is known, it remains essential that the proper means for producing desirable modifications be understood; and furthermore, that the limits of variability, which if exceeded will endanger the plant as a whole, be respected.

Summary

LETTUCE EXPERIMENTS.—1. The top-root ratio varied with experimental conditions between the extremes of 0.79 (table IV, lot 1) and 12.96 (table III, lot 7).

2. Under the following circumstances the top-root ratio was increased: Application of superphosphate either alone or with raw sulphur on a fertile fine sandy loam soil (table I); progressive improvement of fertility in a very poor medium sandy loam soil (table II); with an acid muck-and-sand soil mixture as compared with a fertile fine sandy loam, neutral in reaction (table III); the addition of both lime and superphosphate to an acid muck-and-sand soil mixture (table III); shortened period of daylight illumination in comparison with either normal or artificially lengthened day (tables IV and V); use of sodium nitrate alone and with superphosphate in conjunction with either long or short-day light treatment (tables IV and V).

3. The top-root ratio was lowered by long-day illumination (tables IV and V).

4. The coefficients of correlation for the separate lots of plants vary widely and show no definite relation, respecting their magnitude, to average

sizes of whole plants, but on the whole are decidedly positive in character. The coefficient of correlation for all lettuce plants taken together as a single group (975 individuals) is 0.521 ± 0.018 .

RADISH EXPERIMENTS.—5. The top-root ratio was higher for plants grown on a soil composed of 5 parts fine sandy loam and 1 part medium coarse sand than for those on soil of same components but with the proportion reversed (table VI).

6. As with lettuce, the top-root ratio was higher for short-day plants and lower for long-day plants in comparison with normal-day plants (table VII).

7. Correlation between top and root was variable in degree but clearly positive in character. The coefficient for all radish plants grouped together (277 individuals) is 0.728 ± 0.019 .

TOMATO EXPERIMENTS.—8. The ratios, whether based on the mass relation of whole top, leaves only, or stem only to the root, increased as the size of the container used was greater (table VIII).

9. Higher ratios obtained consistently (though the differences are not mathematically certain) for unpruned plants, for non-deflorated plants both pruned and unpruned, and for plants provided with bottom heat (tables IX, X).

While none of the differences between the coefficients of correlation for the several experimental lots is great enough to be mathematically significant, it is noteworthy that the coefficients are apparently higher for unpruned than pruned plants, for plants from which flowers were removed than those not treated so, for plants grown in larger containers, and also higher as between root and stem than between root and either whole top or only the leaves. The coefficients are more consistent than for either the lettuce or radish plants and the positive nature of correlation even more evident. The coefficient of correlation with all tomato plants thrown into one group is for whole top and root (440 individuals) 0.885 ± 0.007 ; stem and root (389 individuals) 0.792 ± 0.013 ; leaves and root (389 individuals) 0.836 ± 0.010 .

GENERAL.—The concrete results that have been presented are not altogether new and different. In the main, they confirm other investigations in showing the great variability possible in the mass relation of top to root, and give credence to the idea of an "ideal balance" obtainable, within limits, by nature in the process of natural plant adaptation and by plant growers to suit their needs and purposes. It is thought, however, that improved methods for the analysis of data of this type, especially use of the geometric mean and the coefficient of correlation, have been employed, and furthermore, that the fact of persistent positive correlation in size of top and root, regardless of the wide variation shown under special conditions, has been demonstrated.

MICHIGAN STATE COLLEGE,
EAST LANSING, MICHIGAN.

LITERATURE CITED

1. CRIST, J. W. Ultimate effect of hardening tomato plants. Michigan State Coll. Agr. Exp. Sta. Tech. Bull. 89. 1928.
2. KELLEY, T. L. Statistical methods. 1923.
3. PEARSALL, W. H. Studies in growth. IV. Correlations in development. *Ann. Bot.* **37**: 261-275. 1923.
4. ————. Growth studies. VI. On the relative sizes of growing plant organs. *Ann. Bot.* **41**: 549-556. 1927.
5. PEARSON, K. On the distribution of the standard deviations of small samples. *Biometrika* **10**: 522-529. 1915.
6. TURNER, T. W. Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. *Amer. Jour. Bot.* **9**: 415-445. 1922.
7. VARRO. Roman farm management. N. Y. 1913. p. 154. Trans. by A Virginia Farmer.
8. WEAVER, J. E., KRAMER, J., and READ, M. Development of root and shoot of winter wheat under field environment. *Ecol.* **5**: 26-50. 1924.
9. ————. Investigations on the root habits of plants. *Amer. Jour. Bot.* **12**: 502-509. 1925.
10. YULE, G. U. An introduction to the theory of statistics. London. 1924. 7th ed. p. 123.

THE MECHANISM OF THE WATER TIGHT DOOR OF THE *UTRICULARIA* TRAP

FRANCIS E. LLOYD

(WITH FIVE FIGURES AND ONE PLATE)

Introduction

Of the various devices found among plants with the function of entrapping animals, none has been more intriguing than that of *Utricularia*. Interesting and complex as are the pitchers of *Sarracenia*, *Darlingtonia*, *Nepenthes*, *Cephalotus*, these are passive in their behavior, aside from the secretion of digestive enzymes [HEPBURN and his co-workers (10)]. Far more remarkable are considered the submersed *Aldrovandia vesiculosa* L. and the land form *Dionaea muscipula*, the leaves of which, when stimulated, respond by closing over the unwary insect or other animal which touches the sensitive hairs (3, 6). *Utricularia*, however, possesses bladder-like traps which are purely mechanical in their action when once set, and entrap small animals in much the same way as does a mouse trap of the old-fashioned kind. The setting, which is automatic, is the result of a peculiar physiological behavior of the walls of the bladder (recently studied by CZAJA). Beyond this, the trapping action is mechanical, the captured animals being hopelessly ensconced within, when death and digestion finally overtake them. As will be seen, the setting of this trap results from negative pressure of water within. The maintenance of this reduced pressure is made possible by the tight closure of the door. Many attempts have been made to explain how this efficiency is attained and it was the unsatisfactory answers to the question which prompted the present inquiry.

The structure of the *Utricularia* trap (or bladder, as it is frequently called) is so well known and has been so often described, that repetition is unnecessary here (see 1). There are numbers of species, which differ in various details, but they have in common the trap structure, and all appear to work in the same way (8, 9, 11). In the present paper attention is confined to *Utricularia minor*. In this species the trap is compressed pear-shaped, with the entrance at the narrow part where it is truncated. The entrance is guarded by a crescentic ridge, the threshold (plate II, fig. 1), against which rests the edge of the door. The hinge of the door is a semi-circular fold attached to the walls of the trap from the inner edge of the threshold on one side where it gives on the wall of the bladder about the opening to the corresponding point on the other side. The structure of the door is such that, when its cells are alive and turgid, it has strong outward

spring, but is kept from springing too far forward by its own shape and emplacement (fig. 1). If cut away from the sides of the trap, it springs so far forward that the entrance is entirely open. The capacity of the door for bending resides in the form of the component cells (plate II, fig. 7), which have been often described. There is a vestibule-like space in front of the door, from the inner surfaces of which extend glandular trichomes. Similar trichomes, but of more varied forms, grow out also from the outer surface of the door; and in addition to these there are (usually) four stiff, pointed trichomes which serve as levers, and may be appropriately called trigger hairs (fig. 1). These are attached obliquely to the outer surface of the door near the middle point of the lower free edge. The part of the door

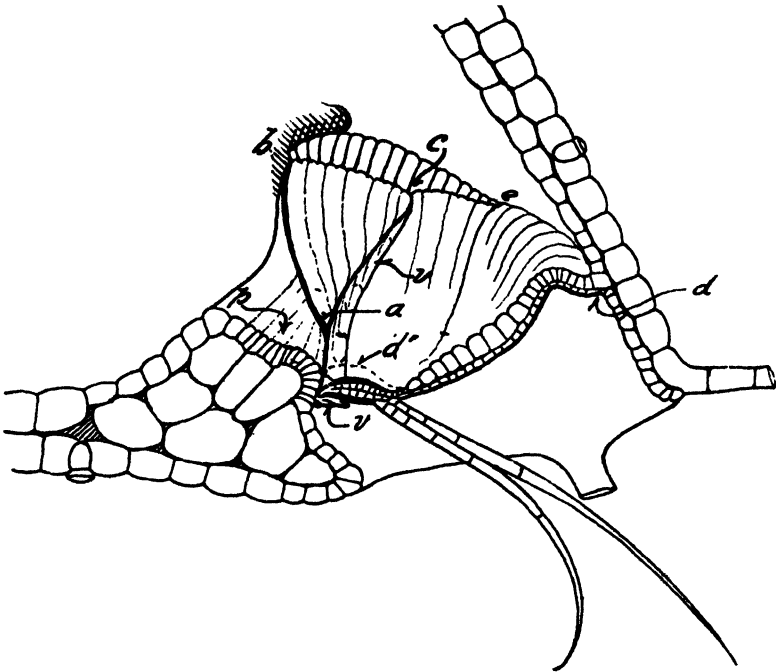


FIG. 1. Diagram of sagittal section and perspective view of the entrance of a trap with the door in the set position and showing the articulation of the door with the wall.

- d e. Transverse reach.
 - b e. Lateral reach.
 - b c. Articulation with the threshold.
 - b a. Lateral reach of the free edge of the door, crossing the threshold.
 - a d'. Middle reach of the door lying in front of the threshold.
- The dotted line below d', shows the tripping position of the door.
- v. The veil or valve.
 - p. The pad.

between the insertion of the trigger hairs and the free edge is well thickened (fig. 1) and has a much thickened cuticle (12); the trigger hairs and the thickened door edge are so firmly of a piece that when the hairs are bent, the door edge bends with them. The fulcrum for this lever movement is furnished by a very thin spot in the door, just above the insertion of the trigger hairs. It must further be understood that the door is domed, the convex surface being directed outwardly. It is thus able to withstand a considerable, but unmeasured, pressure of water. This pressure results from the circumstance that the water within the bladder is pumped out by the walls, the lateral of which consequently become incurved, as it were collapsed, when the trap thus becomes set. It is to be especially remarked that the set condition is permanent (12, 6, 13), from which it must be concluded either that the door does not allow any leakage of water into the interior, or that the cells of the walls can pump it out as rapidly as it gets in.

The foregoing statement of the case is common knowledge and may be summarized briefly as presently follows.

(a) The normal, that is, undamaged or not otherwise discommoded trap, is, when set, water tight as respects mass movement of water (12, 6). For only a brief period (less than 1/16 sec.) can water enter. This occurs when the trap is sprung.

(b) The set condition results from the withdrawal of water from the interior of the bladder or trap, through the walls. There is thus set up a reduced pressure of water within. In this condition the lateral expanses of the wall of the trap are strongly inbent, so that they become markedly concave (2, 12, 6, 13).

(c) When in this condition, the concave sides of the trap can act as relatively powerful springs. By their sudden bulging out, they can pull through the entrance a considerable column of water with great suddenness. Any animal not too large, which happens to lie within this column of water, is immediately carried into the trap.

(d) The sudden bulging out can occur normally only if the trap is sprung (12, 6). This happens when the trigger hairs are suitably disturbed, namely, in such fashion that the middle portion of the free edge of the door is lifted. At the moment of lifting, the side walls spring out, the whole mechanism passing instantly from a state of labile into one of stable equilibrium.¹

(e) The walls hereupon begin to pump out the water at such a rate that the trap may become set again after the lapse of 15 minutes (12) or usually

¹ Springing of the traps occurs when the plant is raised out of the water into the air and the water allowed to drain off. BROCHER noticed a clicking sound caused thereby. MERL's explanation that the surface tension of the films of water about the trigger hairs accomplishes the tripping is probably correct.

a rather longer period (13). We (LLOYD and R. D. GIBBS) have observed the capture of a second worm 35 minutes after the first capture. As soon as a sufficient volume of water has been pumped out, that is, when the sides of the trap have become more or less concave, the trap has become automatically reset.

(f) The pumping out of the water is a function of the walls, which CZAJA described as semipermeable. The action is referred to the two- and four-armed trichomes, which are regarded as absorbent structures (12, 6). There is insufficient proof of this, but the matter does not enter into the present discussion.

(g) As stated in (a) above, the set condition is permanent. MERL saw that no dyes enter even during a period of days. CZAJA showed, by passing a human hair underneath the edge of the door, that unless the opening at the entrance is completely closed, the trap does not reset itself.

(h) This hermetically sealed condition, to use MERL's phrase, is owing, according to all observers except BROCHER and WITHEYCOMBE, to the firm pressure of the lower free edge of the door against the top of the threshold. According to the majority view the effective sealing of the entrance results from the pressure, eventually produced by the turgor of the door cells, of the free edge of the door against the opposed surface, but seconded by the presence of mucus (13) or mucilage which acts as a plugging material, filling the reentrant angle formed at the edge of the door. All students of the matter have agreed on this latter point. The various views with regard to the articulation of door edge with the threshold are indicated by the following passages: ". . . ihr freies Ende auf einem hufeisenförmigen Rahmen als Widerlager ruht" (8, 1889, p. 152); ". . . mit der freien vierten Seite dem den Antennen gegenüberliegenden dicken, mit ebenfalls Schleim bildenden Zellen besetzten Mündungsrande von unten d.h. vom Blaseninnern her, anliegend" (4, 1888, p. LIX); "ihre Klappe schliesst mittels eines Schleimwulstes so fest, dass aus dem Innern nichts heraustreten kann" (11, 1910, p. 211). "Die höchst elastische Klappe die . . . dem Widerlager ziemlich fest aufliegt . . ." (12, 1922, p. 73). "Der mittlere Teil liegt dem Polster auf und ist schmal, die seitlichen Teile liegen ebenfalls auf . . ." (6, 1922, p. 713); BROCHER, sensing the unsatisfactory character of these or similar descriptions, remarked "s'il était disposé aussi simplement que cela est représenté généralement dans les livres, cet état de pression négative ne pourrait exister, et se maintenir, à l'intérieur de l'utricule. Mais tel n'est pas le cas. La disposition est un peu différente et rend cet état de chose possible." He then states that the inward flexing of the sides has the effect of augmenting the curvature of the door in such fashion that "le bord libre de celui-ci s'applique d'autant plus exactement contre le re-

bord (X in his figure) de l'utricule qui lui est opposé, que la courbure de l'opercule est plus considerable" (2, p. 43-44). "This margin (of the valve or door) is sharp, thin and smooth and rests on the edge of a rim or collar which projects into the interior of the bladder. The collar *obstructs any outward movement* (italics mine) with the result that the valve (door) can only open inwards" (ARBER, 1920, p. 93). The last quotation is given, not in criticism of its author, but because it summarizes the then accepted view.

WITHYCOMBE (13), however, became still more aware of the discrepancy between the accepted accounts and the behavior, and came to believe that the edge of the door rests, not on the top of the threshold, but in a "slight groove" in front of the "collar cells." His evidence, however, derived from fixed and paraffin-sectioned material, misled him on this point, the threshold tissues having shrunk so much as to completely distort the shape of the upper surface. As will be shown, the door edge rests against the top of the "collar cells," or pad, and not against their forwardly directed lateral faces. WITHYCOMBE did not apparently apprehend the fact that the door edge crosses the threshold (6). The sealing "mucus" he supposed to have been secreted by the middle layer of the "collar cells" (his figure 5, 13). WITHYCOMBE, therefore, believed rightly that the door edge presses inwardly against an outwardly opposing surface, but failed to determine what surface, and further accepted mucilage or mucus as the sealing material.

Having thus been led to investigate the matter, it has been possible to show that the mechanism which prevents the inward leakage of water into

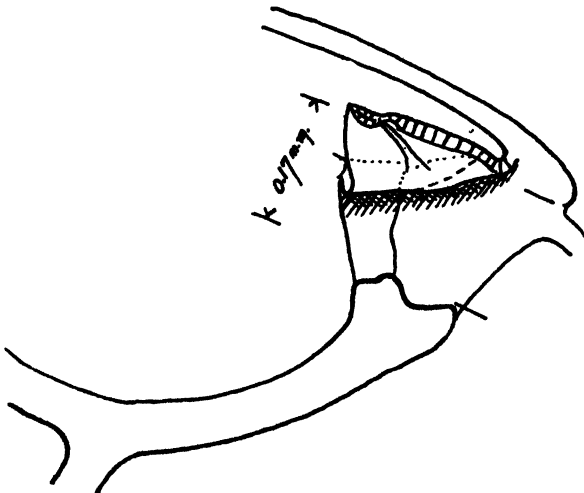


FIG. 2. Position of the door when widely opened.

the trap is not the door alone with a seal of mucilage, but the door acting in cooperation with a valvular membrane attached to the front margin of the threshold. Furthermore, the lower edge of the door lies, when in normal position, not on the threshold, nor pressing outwardly against the outer raised margin, but *pressing inwardly against the outer aspect of this margin*. It is this emplacement of the free edge of the door along a *ligne d'appui*, coupled with the valvular action of a *curtain-like membrane*, that together prevent the seepage of water. The permanence of the set condition of the trap, depending on the foregoing, has been verified by me, with the cooperation of Mr. R. D. GIBBS. In addition we have also determined the behavior of the traps, finding ourselves in agreement with CZAJA and MERL especially in regard to the springing of the trap. We have done this with a needle point and have watched animals (small worms) being entrapped,² and have come to the conclusion that the downward movement of the trigger hairs (that is, away from the plane of the antennae) is the most effective, if not the only normal condition for the release of the door. I find that unless the lower edge of the door is engaged by pressing inwardly against the raised outer margin of the threshold (figs. 1, 4), it is unable to resist the pressure of the outer water (assuming the trap to be set) and accordingly bends inwardly, admitting water, or air if water is not present, as BROCHER observed. To release the door edge from this engagement, it must be lifted free of the opposing margin of the threshold. The moment this is accomplished the door gives way entirely to the intruding water. It is now in order to present an account of the mechanism as thus comprehended. The significance of a number of details of structure hitherto negligible, or at any rate neglected, will now come to light.

Observations

First we will take up the opening into the trap and the threshold. Considering, for the purpose of description, that the antennae are attached to the *upper* lip of the trap, the threshold bars the entrance a short distance within the vestibule. The plane in which it lies will be used as a plane of reference in the following description.

THE THRESHOLD

The threshold is a massive curved structure, the upper surface of which forms a flattened crescent with the horns projecting slightly toward the interior (figs. 1, 4; plate II, figs. 1, 2, 9). Looked at from above, the eye

² A motion picture has been made of this, using no artificial assistance, depending wholly on the behavior of the worm. This picture was exhibited at the New York meeting of the Botanical Society of America, December, 1928.

of the observer being in the threshold-plane, the forward or outward margin is curved like a conventional Cupid's bow, the chief curve, that of the body of the bow being in the middle, the rear or inner margin, straight or nearly so. The inner surface extends steeply downwards and is clothed with bifid glandular or absorbing hairs, whatever this may mean. There appears, however, to be another margin within this one just described. This is due to the presence of a pad or mat of epithelium composed of glandular trichomes (13) (trapezoidal as seen from above) which in a transverse section of the threshold appear columnar with rounded free surfaces. The shape of the pad is such that the rear limb is symmetrical with the forward limb, being broad near the horns and in the middle (though here not as wide as the total width of the threshold) and narrow between these points (plate II, fig. 9). The relation of this pad of tissue to the threshold as a whole is important and may best be understood by viewing a transverse section of the threshold (sagittal with reference to the trap as a whole). In such a section (fig. 5; plate II, figs. 3-5, 8) one sees the pad as a dense tissue forming the top of the threshold and bending over the outer margin. This bent-over part furnishes a surface for the free edge of the middle portion of the door to rest against when in a set position. As above stated, this margin is outwardly curved. To this curve fits the curve of the middle portion of the free edge of the door when in the set position. It may be observed that the raised outer marginal epithelium with its pad of glandular cells is supported by very large cells jutting upwards above the general level of their neighbors.

The pad ("Pflasterepithel" as GOEBEL called it) is of further and prime importance, in that *the cuticle of its glandular cells exfoliates* during development, and before the trap opens for business, *but remains attached by its forward margin*. This loosened cuticle thus forms a veil suspended between the horns of the threshold and attached to it along a line well below its forward margin at the forward limit of the pad. The presence of this veil has been hitherto overlooked; indeed, I have overlooked it myself for years. It is only with great care that sections can be cut (of fresh material, of course) without tearing it away. When it remains, it is so diaphanous that either it is not seen (in transverse sections of the threshold usually) or appears merely as the top of the threshold when seen looking along the sagittal axis. The forwardly directed surface of the pad and the veil form a pocket. *It thus comes to have a definite function as a valve, sealing the fissure between the edge of the door and the threshold*, just as a bit of leather stops the leakage of the piston head of a pump.

The exfoliation of the veil takes place rather late in the development of the trap. In bladders as small as 0.33 mm. in length it has not yet appeared

but is recognizable at 0.47 mm. At these stages of development the door overhangs the threshold (GOEBEL) coming into its definitive position later. When first evident, it is to be observed over the outer one or two rows of pad cells, the exfoliation evidently beginning in this region and extending back (plate II, figs. 3-5, 8). In younger traps the veil appears more definite and circumscribed, but with age becomes more extensive and ill-defined at its loose posterior border. The ragged appearance of the loose margin of the veil is due to its irregular tearing away from the pad cells in the more interior region, that toward the inner margin of the threshold. The very thin ragged portion bears the evident imprint of the tops of the cells from which it arose.

A feature of the development of the veil must be especially mentioned, namely, that during the earlier period of its exfoliation there is an expansion of its area, so that it balloons away from the cells from which it arises. The impress of the cell top is, as a result, an exaggeration of the pattern present in a surface view of the pad. The large size of the meshes of the veil appears out of congruity with the smallness of the pad cells; it is only when the behavior during origin is known that the incongruity is removed.

The veil is cutinized (is insoluble in strong sulphuric acid) and yellows with iodine, when it can be easily seen. When bleached of iodine and lying in glycerin it becomes exceedingly diaphanous.

THE DOOR

The door, called by some the valve, is a structure of complex curvatures. It may best be described in relation to figures 1 and 3. Figure 3 is introduced for the further purpose of indicating my dissent from C'ZAJA's mapping of the areas which he recognizes. His effort was concentrated on explaining the behavior of the door as a structure which presses down and outwardly on the threshold. Since this is not what happens, his description fails in cogency.

The insertion of the fixed margin of the door is along a U-shaped line, the plane of which is almost at right angles (*ca.* 100°) to the threshold plane, and so disposed that the free ends of the U coincide with the ends of the threshold where it gives on the side wall (fig. 1, *b c*). From this line of insertion the door hangs in such fashion that the free edge, that which articulates with the threshold, is nearly circular. This shape is retained normally by its own turgor. Plate II, fig. 6, is a photograph taken at right angles to the plane embracing the free edge of a freshly removed door. When in position, the thin edged sector (*a b c*, *a'b'c'*, fig. 3) is somewhat bent inwardly in adjusting itself to the threshold. In addition to this curvature, the door is also curved normally to the plane of the free edge. When

forced by pressure into a nearly flat plane, it takes the form of fig. 7, plate II. There are, it is evident, as indicated in fig. 3, three regions—a middle generally circular area, truncated below to form the middle region of the free edge ($a a'$), and flanked by two triangular smaller pieces ($a b c, a' b' c'$) which fold along the lines $a c$ and $a' c'$ when the door is in position. The door is attached along $b c d c' b'$, the hinge at d being a strong curve which

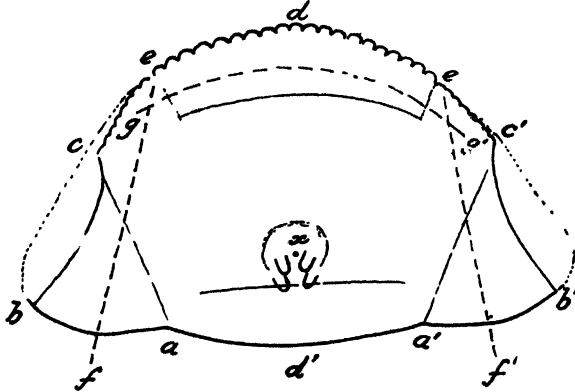


FIG. 3. Map of the door as seen when approximately flat.

- $b a a' b'$. The free edge of the door.
- $a a'$. Middle reach.
- ab and $a'b'$. Lateral reaches.
- ac and $a'c'$. Lines of the fold.
- $c a a' c'$. Follow the curve of the threshold when the door is in the natural set position.
- $e f$ and $e' f'$. Approximate lines of lateral flexure.
- $g g'$. Approximate line of transverse flexure. Median flexure occurs along the line $d d'$.
- x . Central hinge area; the base of the four trigger hairs are indicated, below which is the thickened margin or wale of the door.
- $b c$ and $b' c'$. Articulations of the door with the threshold.
- $c e$ and $c' e'$. Continuations of the lateral articulations against the wall of the trap.
- $e d e'$. Transverse articulation.

is maintained in the transverse reach $g g'$ to become in the lateral reach ($e c, e' c'$) a simple attachment. The structure of the wing $a b c$ is continuous with that of the middle piece, and I am unable to identify the triangular areas plotted by CZAJA ($e f g$ in his fig. 3, 1922, my fig. 3). The line of the bend, with which CZAJA seems to identify his triangle $e f g$, lies strictly along $a c, a' c'$ in my fig. 3 ($e d$ in his figure). When in position this line of bending follows the forward edge of the threshold from the horn (c, c' , fig. 4) to a point on this edge where the free edge of the door crosses (a, a' , figs. 1, 4) to the outer surface of the threshold. The chink formed between the

fold of the door and the edge of the threshold lies behind the veil, which accordingly blocks it. CZAJA's account postulates a bend running above the thickened margin of the middle piece ($f f' e'$ in his figure), whereas as a matter of fact there is none there. He accords a corresponding discontinuity of structure indicated in his figure by lines. The fact seems rather to be that the structure of the lateral areas is completely congruous with that of the middle piece.

The narrow region below the insertion of the trigger hairs is much thickened and has a thick cuticle. The shape, as seen in section transverse to the run of the edge, is lenticular, the edge which rests on the outer surface of the threshold being rather sharp. Just at and above the insertion of the trigger hairs the door is very thin. This thin area is nearly circular and constitutes a hinge, allowing the thickened edge of the door to bend up and inwardly so that it can come just above the resisting edge of the threshold. When this condition is established, the door has little resistance and flaps inwardly, permitting an inrush of water. This concluded, it springs back into position, the stiff edge sliding over the pad till it regains its *ligne d'appui*. In doing so it pushes before it the loose veil which automatically comes into position as a valve not only near the horns of the crescentic threshold, as above stated, but along the whole length of the edge of the middle piece as well.

It is difficult, indeed, almost impossible to cut a section of fresh material so that the door remains in exactly the right position. A half-door inevitably curves free of the edge and lies more or less above the threshold, the veil then looking like a ragged mass of stuff not calculated to attract attention. I have accordingly studied the silhouettes of entire fresh traps making negatives with long exposures. These, when examined, usually show a shadow made by the veil, and the door in its proper position. Once one knows what to look for, it is quite possible, often easy, to see the veil in the living trap by looking obliquely into the entrance with a low power objective. In some cases even the quasi-cellular structure can be seen (plate II, figs. 1, 2, 10).

Little has been said yet about the hinge of the door as a whole. In the transverse reach this hinge is a strong flexure toward the inside of the trap, bending back again into the curvature of the middle piece. As the transverse reach fades into the lateral reaches, the bend of the hinge becomes less pronounced, until it merely curves downwards and a little away from the wall. In the region over the end of the threshold a mass of tissue lifts the end of the door (marked $b c, b' c'$, fig. 1) away somewhat from the wall, a group of large cells, a sort of buttress, causing this (c, c' , fig. 1, 4). This grades in the forward direction into the wall and offers attachment to the

stretch of the arch *b* to *e*, fig. 1. At point *e* there occurs a slight angle, breaking a little the continuity of the arch, the point at which the strongly bent hinge cells (*e d e'*) begin.

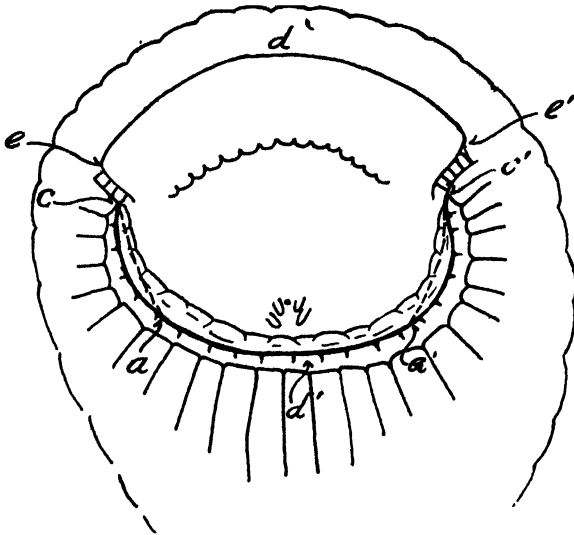


FIG. 4. Diagram of the entrance from the inside of the trap.

The behavior of these regions of the door along the arch of attachment may be seen by studying the silhouette of a trap which has swallowed a glass rod (fig. 2). A trap freed from the plant will, when the end of the glass rod is pushed against the trigger hairs, jump forward and swallow the end of the rod, as CZAJA found using a needle. I found that a rod 0.1 mm. in diameter is easily swallowed. If the rod is tapering, it may be pushed forward, with precautions to do no damage to the entrance mechanism of the trap, till the door is opened to its maximum. When in this position the rod was 0.17 mm. in diameter, representing approximately the maximum possible displacement of the door, probably more than normally occurs. The middle piece of the door (*f*, *e*, *e' f'*, fig. 3) suffers maximum displacement, the sides of the door being bent upwards approximately along the lines *e f* and *e' f'*, fig. 3. This bend is most pronounced toward *f*, and towards *e* gradually fades into the curvature between *e* and *e'*, though not without a jog at these points. The tissues along *e-f* and *e'-f'* are thus strongly pulled out of equilibrium and tend to straighten back in a direction normal to *e-f* and *e'-f'*. The cells at right angles to the line *g-g'* tend to bend back normally to this line. It is certain that the cells along *b c e* and *b' c' e'* are displaced from position only very slightly, merely enough to accommodate themselves to the sharp upward bend along *e f* and *e' f'*.

What the maximum displacement of the door is when it opens by displacement by the water column pressing against it cannot precisely be said, but, since (in the trap I measured) the maximum opening has the transverse diameter of 0.17 mm. and the same trap immediately, on springing the door, swallowed a rod 0.1 mm. in diameter, it must open more widely than has been supposed. MERL speaks of a lunate "halbmondförmig" slit. Any opening short of a good-sized throat could not engulf the large objects often found in the traps; and it must not be forgotten that during the time the door is open, less than $1/16$ of a second, a column of water fully 1 mm. long passes in. During the movement of this column the door must remain stationary in a position presenting a throat of diameter greater than 0.1 mm. To do this the sides of the door must be bent upwards, approximately, though not as much, as in fig. 7.

The recovery of the set position of the door does not wait on the complete relaxation of the lateral walls, and in fact probably always closes before. This is shown by the fact that, after any event of tripping, the walls will on puncturing the bladder with a sharp needle relax further to the maximum possible extent (12). The fact is used here to emphasize the vigorous action of the door, which recovers its position in spite of a still inflowing column of water. The change in shape of the trap after tripping with a needle and after puncturing with a needle was illustrated by MERL.

Flexure of the door also occurs along the line $d d'$ in the sense opposite to the flexure $e f$ and $e' f'$. The great thickness and elasticity of the door

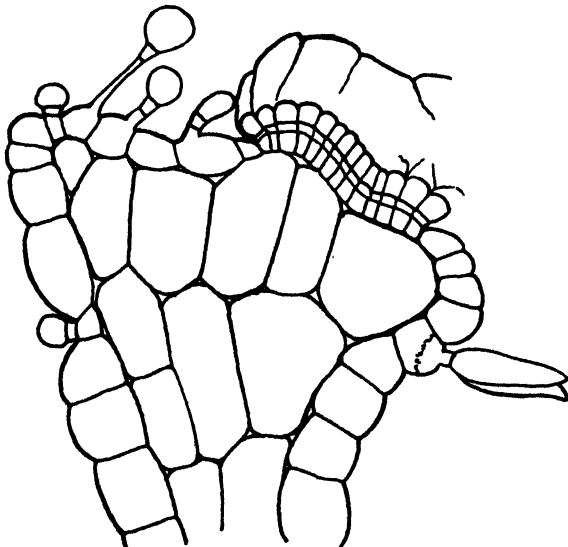


FIG. 5. Transverse section of the threshold showing the veil when fully developed.

edge, forming a wale of tissue reaching from a to a' , enable it, while bending before the moving column of water, on release of pressure to immediately resume its semicircular form, bringing it back into its original position, the whole edge from b to b' (fig. 3) being in the form of an unbroken arch (plate II, fig. 6). This wale of tissue is important, therefore, in bringing the door into the set position. Its initial flexure, due to the leverage by the trigger hairs, is at its middle point (d'). The pressure of the water causes it to arch in the opposite sense, the flexure spreading outwardly along the radii from the centre x .

Correlated with the remarkable degree of flexure possible, and with the strong resistance of the door to deformation, is the form of the cell walls. These are never straight, but curved like so many springs. This is true of both radial and periclinal walls (plate II, fig. 7). This is, of course, a matter of common observation. What is important for the correct understanding of the working of the door is the proper evaluation of the rôle of the thickened wale along the middle reach of the door edge. This acts by swinging into position, engaging the outer edge of the threshold as a catch which in spite of its firm engagement can be easily released by downward pressure of the trigger hairs. Its almost cartilaginous consistency is due in large part to the much thickened outer walls (MERL) of the very small cells.

Summary

It has been shown in the foregoing that when the trap of *Utricularia minor* is in the set position, the middle part of the free edge of the door rests against the outer surface of the pad of glandular cells of the threshold, and not, as heretofore supposed, against the top or any part of the top or against the edge of the pad. It maintains its position against water pressure by virtue of the resistance offered by the threshold. The outer reaches of the door edge cross obliquely the sides of the threshold. There is of mechanical necessity, therefore, a fold between the middle and lateral portions of the door which would, if not otherwise provided against, allow inflow of water under the reduced pressure known to be present in the trap when set. This possible leakage of water into the interior of the trap is prevented by the presence of a veil or curtain attached to the outer surface of the threshold and stretching from one horn to the other of the crescentic threshold in such a manner as to act as a valve to exclude water by pressing on the edge and folds of the door. This veil, or valve, is the outer cuticle released from the cells of the pad, being set free from the inner margin of the pad and remaining firmly attached in front. The veil shows the imprint of the top of the cells from which it sprung. The earlier formed part of the veil suffers considerable extension, or ballooning, recalling the

behavior of the cuticle in certain other glandular trichomes (*Pelargonium*). The veil arises during the later period of development of the trap, so that when the trap begins to function as such, the veil is ready in position.

McGILL UNIVERSITY,
MONTREAL, CANADA.

LITERATURE CITED

1. ARBER, A. Water plants. Cambridge University Press. 1920. (p. 91.)
2. BROCHER, F. Le problème de l'Utriculaire. Ann. de Biol. Lacustre. 5: 34-46. 1911.
3. BROWN, W. H., and SHARP, LESTER W. The closing response in *Dionaea*. Bot. Gaz. 49: 290-302. 1910.
4. BÜSGEN, M. Ueber die Art und Bedeutung des Thierfangs bei *Utricularia vulgaris* L. Ber. d. bot. Ges. 6: LV-LXIII. 1888.
5. COMPTON, R. H. The morphology and anatomy of *Utricularia brachiata* Oliver. New Phyt. 8: 117-130. 1909.
6. CZAJA, A. TH. Die Fangvorrichtung der Utriculariablase. Zeitschr. Bot. 14: 705-729. 1922.
7. DARWIN, C. Insectivorous plants. 1875.
8. GOEBEL, K. Pflanzenbiologische Schilderungen. Marburg. 1889.
9. ———. Morphologische und biologische Studien V. *Utricularia*. Ann. Jard. Buit. 9: 41-119. 1891.
10. HEPBURN. The biochemistry of the American pitcher plants. Trans. Wagner Free Inst. Sci. Philadelphia, 11: 1-95. 1927.
11. LUETZELBURG, P. VON. Beiträge zur Kenntnis der Utricularien. Flora 100: 145-212. 1910.
12. MERL, E. M. Biologische Studien über die Utriculariablase. Flora 115: 59-74. 1922.
13. WITTHYCOMBE, C. L. On the function of the bladders of *Utricularia*. Jour. Linn. Soc. 46: 401-413. 1924.

EXPLANATION OF PLATE

FIG. 1. Entrance of trap viewed from the outside showing the veil and the upper surface of the threshold. The door is sprung outwardly by its own turgor, but it is so seen because a part of it has been removed in sectioning the trap.

FIG. 2. The veil and front edge of the threshold of another trap. The lower edge of the door can be seen again in the sprung position.

FIG. 3. Section of the threshold of a young trap showing the exfoliating cuticle at a rather early stage.

FIG. 4. A somewhat later condition.

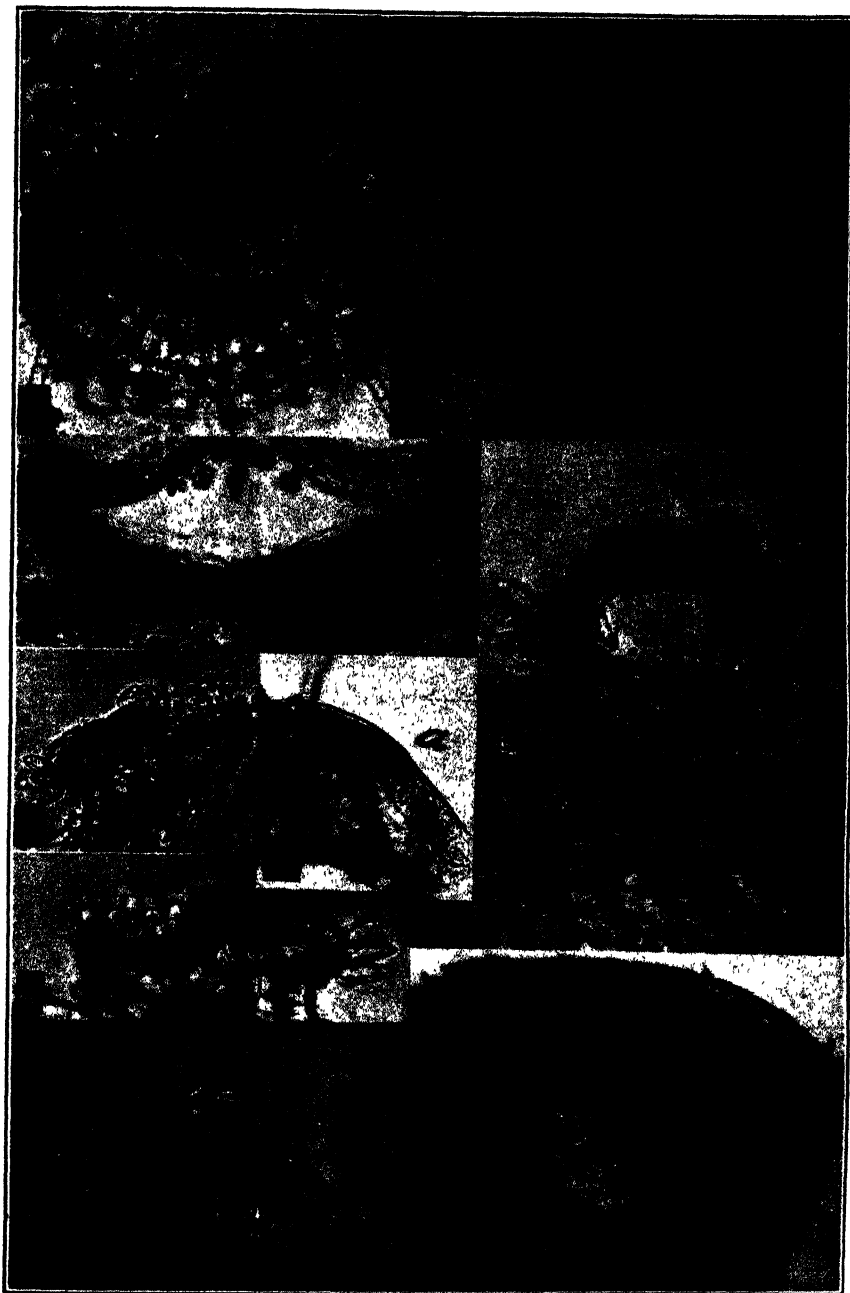
FIGS. 5 and 8. The fully developed veil as seen in transverse section of the threshold.

FIG. 6. Half of the door showing the thick wale above the middle reach of the lower edge and the thin lateral portion which crosses the threshold from *a* to *b*—text fig. 1.

FIG. 7. A half-door laid out nearly flat viewing the inside surface. Letters as in text fig. 1.

FIG. 9. View of a little over half a threshold seen from above. The anterior margin bears the veil. The inside face of the threshold (above) bears glandular trichomes. The trapezoidal cells of the pad can be seen. The dark patches are caused by air in the intercellular spaces, here as in other figures.

FIG. 10. View of the door and veil in the set position as seen when looking into the threshold of a living set trap.



LLOYD-UTRICULARIA

SOME CRITICAL COMMENTS ON THE METHODS EMPLOYED IN THE EXPRESSION OF LEAF SAPS¹

BERNARD S. MEYER

(WITH ONE FIGURE)

In many types of plant physiological research, accurate determinations of the physico-chemical properties of expressed plant saps are important. In undertaking such determinations, however, the technical methods of collecting and treating the samples and of expressing the saps have not always been judiciously chosen to meet the requirements of the investigation. Such methods have sometimes been followed according to blind prescription with little or no regard for the limitations imposed upon them by the physiology or anatomy of the tissues. The following comments upon the technique of such determinations, and the attempted critical evaluation of the more generally used methods are based upon the experience gained in an extended investigation upon the physical and chemical properties of expressed leaf saps. The discussion in this paper refers primarily, therefore, to leaves and leaf saps.

The earliest investigators of the properties of expressed plant tissue fluids carried out their determinations upon saps expressed from living, untreated tissues. ANDRÉ (1) appears to have been the first to observe that fractions of sap successively expressed from an untreated leaf sample showed progressively increasing concentrations of solutes as measured by the depression of the freezing point.

DIXON and ATKINS (2) obtained results similar to those of ANDRÉ when they expressed sap from untreated plant tissues. They explained the progressive increase in the osmotic concentrations of successively expressed fractions of sap by assuming that a larger number of cells burst at the higher pressures, thus permitting solutes to escape to which the protoplasmic membranes of the unbroken cells at the low pressures were impermeable.

Since this differential expression of sap from untreated tissues makes it practically impossible to express from them representative samples of sap, DIXON and ATKINS introduced the method of freezing the leaves in liquid air before subjecting them to pressure. They presented data to show that the successively expressed fractions of sap from a leaf sample frozen in liquid air did not differ materially in osmotic value. They also found that the volume of sap expressed from frozen leaves was larger than could be expressed from unfrozen leaves, and was freer from debris.

¹ Papers from the Department of Botany, the Ohio State University, no. 221.

GORTNER and HARRIS (5), also principally interested in determinations of the osmotic values of expressed plant tissue fluids, substituted the ice-salt bath as a more universally available mode of freezing. In this method the tissues to be frozen are placed in a glass tube of suitable size, which is then stoppered and immersed in an ice-salt bath in which a temperature of -15°C. to -20°C. is usually maintained. The usual field precautions to be observed with this freezing method are discussed by its originators. A number of other investigators have employed this method.

GORTNER, LAWRENCE, and HARRIS (6) checked the conclusion of DIXON and ATKINS that the concentration of solutes in samples of sap successively expressed from unfrozen leaf samples showed a progressive increase. They expressed the sap from untreated leaf samples from a number of species of plants and found some in which successively expressed portions of the sap showed decreasing concentrations of solutes, and others in which all of the fractions of expressed sap showed approximately the same concentrations of solutes. Although their results deviate from those obtained by DIXON and ATKINS, they do not invalidate the principle that sap samples expressed from unfrozen tissues cannot be taken as representative of the conditions existing in the tissue from which they have been exposed.

Methods of freezing leaf samples in solid carbon dioxide have been employed by HARVEY (7), LEWIS and TUTTLE (10), MEYER (11), and others.

GAIL (3) ground the leaves of western conifers through a fine meat grinder before expressing the sap. Some samples were also frozen but GAIL reported that there was little difference between the osmotic value of the samples merely ground, and those which were both ground and frozen.

NEWTON, BROWN, and MARTIN (12) have recently described a method of finely grinding plant tissues and expressing the fluids at low pressure which they believe results in the expression of a sap which represents substantially the composition of the original tissue fluids. All operations were carried out at a temperature close to 0°C. Since the object of this method is to obtain the cell contents in as nearly unchanged conditions as possible, freezing of the tissue is not permissible because of its known precipitating effect upon proteins. This method promises to prove of value and general application in indicating the distribution of any constituent of plant tissues between the physiologically active and the inert portions of the tissues.

GOLDSMITH and SMITH (4) appear to have been the first to rely upon the method of treating leaf samples with a toxic vapor in an extensive investigation of the physico-chemical properties of plant saps. In a recent investigation they used chloroform vapor treatments upon the leaves of the Engelmann spruce (*Picea engelmanni*) with apparently satisfactory results. The leaves were exposed to the vapor for thirty-six hours before the saps were

expressed, the investigators relying upon refrigeration during this period to prevent any appreciable enzyme action. Freezing point depressions obtained with saps expressed after this treatment were corrected for the solubility of chloroform in water. This method was checked against the ice-salt freezing method for leaves in the summer condition and excellent agreement found in the results, but apparently it was not checked against other methods while the leaves were in the winter condition. The subsequent discussion in this paper shows that such a check would have been desirable. However, there is no indication in the results obtained that this method is not effective with winter-hardened leaf tissues.

During the past several years the writer has made a number of comparative studies of the effects of several methods of treating leaf samples upon the amount of sap which can be expressed from them and the osmotic value of the sap. Parallel series of determinations were made at different seasons for the leaves of several evergreens in the living condition, after freezing in an ice-salt bath, after freezing in solid carbon dioxide, and after grinding to a shredded pulp.

Table I records the results of a representative series of determinations upon the 1927 leaves of the pitch pine (*Pinus rigida*). Similar results were obtained in every other series of determinations made.

TABLE I

EFFECT OF VARIOUS TREATMENTS OF PITCH PINE LEAVES UPON THE AMOUNT OF SAP WHICH CAN BE EXPRESSED, AND UPON THE OSMOTIC VALUE OF THE EXPRESSED SAP
PRESSURE = 5,000 LBS. PER SQ. IN.

DATE	MOISTURE CONTENT	DETERMINATION	UNTREATED	FROZEN (ICE-SALT)	FROZEN (CO ₂)	GROUND
Aug. 3, 1927	<i>per cent.</i> 59.4	Sap expressed (cc. per 100 gm.)	23.9	44.3	47.6	42.9
		Osmotic value (atmospheres)	9.40	15.20	15.91	18.68
Jan. 22, 1928	54.7	Sap expressed (cc. per 100 gm.)	17.7	15.0	41.0	31.3
		Osmotic value (atmospheres)	12.41	13.04	21.12	25.73

An analysis of these data brings out a number of facts which are pertinent to the present discussion. The water content of the leaves was 4.7 per cent. higher in August than in January, a fact which may be expected to have a minor influence on the volumes of sap which could be expressed.

In the summer approximately the same volumes of sap could be expressed from samples frozen in an ice-salt bath, from samples frozen in

solid carbon dioxide, and from samples shredded through a meat grinder. A much larger volume of sap could be expressed from all the treated samples than from the untreated sample.

The summer osmotic value of the sap expressed from the ground sample was about three atmospheres higher than the osmotic value of the sap expressed from either of the frozen samples, which checked closely with each other. A much higher osmotic value was obtained with all the treated samples than with the untreated sample. The higher value shown by the sap from the ground sample was perhaps due to the favorable conditions for oxidations and other chemical changes which probably occur in the tissues during and immediately following the grinding process.

In the winter, only from the sample frozen in solid carbon dioxide was a volume of sap expressed which compared with the volumes expressed during the summer from all the treated samples. The volume of sap expressed from leaves frozen in an ice-salt bath was of the same order of magnitude as the volume expressed from the untreated leaves. The volume expressed from the ground leaves was distinctly smaller than the volume expressed from leaf samples frozen in solid carbon dioxide.

The winter osmotic value of the sap expressed from the leaves frozen in an ice-salt bath was not appreciably different from that of the sap expressed from unfrozen leaves. The sap expressed from the leaf sample frozen in solid carbon dioxide showed a much higher value, and that expressed from the ground leaf tissue was still higher, but the last two values were obviously of the same order of magnitude.

It is believed that the difference between the results obtained with leaves in the summer condition, and the results obtained with leaves in the winter condition is due principally to an increase in the amount of "bound water" in the leaf tissues during the winter months. In a previous paper (11), the probable relationship between seasonal variations in the bound water content, and seasonal variations in the hydrophilic colloid content of the leaves of this species has been indicated. During the summer months it appears that only a relatively small amount of the water present in the tissues of pitch pine leaves is bound. The methods of freezing in an ice-salt bath, freezing in solid carbon dioxide, or grinding, are therefore all effective in increasing membrane permeability, presumably through disorganization of the protoplasm, and thus permit the expression of a representative sample of sap.

During the winter months, however, a relatively large proportion of the water present in the tissues apparently is in the bound condition. The ice-salt freezing method does not have a drastic enough effect upon the tissues to permit the expression of a representative sample of sap. Presumably

this is because the molecular forces binding the water are of sufficient magnitude to prevent the occurrence of any appreciable amount of crystallization of water in the tissues. At this temperature, according to all present conceptions of the mechanism of ice crystallization in plant tissues, crystals ordinarily form only in the intercellular spaces, withdrawing water from the cells as they elongate. The sap expressed is therefore similar in amount and properties to that expressed from untreated leaves.

At the temperature of solid carbon dioxide (-80° C., and lower), the forces of crystallization are undoubtedly of sufficient magnitude to overcome the molecular forces binding water. Leaves are usually frozen solid by this treatment, indicating that most of the water present in the tissues freezes within the cells. The result of this treatment is that representative samples of sap can be expressed under pressure.

The grinding treatment also permits the expression of representative samples of sap at all seasons of the year. The effectiveness of this treatment is apparently due to the disruption of the physical relationship existing between the bound water and the tissues, through the bruising and tearing action which the grinding process exerts on them.

The writer (11) has already called attention to the discrepancy between the results obtained by GAIL (3) and those obtained by KORSTIAN (9) in their investigations of the winter osmotic values of the expressed leaf saps of certain western evergreens. The divergent results obtained by these two investigators are apparently due to different methods of treating the leaf samples. KORSTIAN froze the leaves in an ice-salt bath while GAIL, as has already been noted, used what was essentially a grinding treatment.

The demonstrated and theoretically expected limitation of the ice-salt freezing method to unhardened tissues makes it desirable that a field method be devised which is not subject to such a limitation. The liquid air freezing method, vapor treatment methods, and grinding method are difficult to adapt to ordinary field conditions. Neither is the spraying method of freezing with solid carbon dioxide, as usually employed, feasible in the field.

Recent developments in the commercial production of solid carbon dioxide² have permitted the development of a field method of freezing leaf samples with solid carbon dioxide which will be briefly described.

The solid carbon dioxide must first be reduced to fragments varying up to the size of a small marble. These crushed fragments are poured into an insulated container³ until a layer about one inch in thickness is deposited

² Solid carbon dioxide is manufactured and sold by the Dry Ice Corporation of America, New York and Chicago, under the trade name of "Dry-Ice."

³ Stanley "Super-Vac" Food Jars (all steel) in the quart size have been found to be very serviceable for this purpose.

in the bottom. A stoppered tube of suitable size (about 8 inches long and 1.25 inches in diameter) is inserted and held upright while the carbon dioxide is poured around it until the jar is filled to a level slightly below the top of the tube. The jar is stoppered and is then ready to be carried into the field.

Leaves are thrust as rapidly as collected into a glass tube of the same size as the one inserted into the jar. When filled the tube is stoppered and substituted for the empty tube in the jar. This particular procedure is necessary because the solid carbon dioxide so rapidly becomes compacted in the jar, that it becomes practically impossible to dislodge the individual particles sufficiently to force the collecting tube into the mass of solid carbon dioxide. The solid carbon dioxide slowly disappears by sublimation, but usually a sufficient amount remains in the jar to keep the leaf samples frozen for about 24 hours.

There is no reason to believe that the method of freezing leaf samples in liquid air as first employed by DIXON and ATKINS will lead to results for sap determinations which are markedly different from those obtained with the carbon dioxide freezing treatment. Although the writer has not had the experimental opportunity to confirm this statement, comparative data from HARVEY (7) support it. For hardened cabbage leaves HARVEY recorded a difference of only 2.3 atmospheres in osmotic value between sap samples expressed after these two treatments. For unhardened cabbage leaves he recorded a difference of 1.7 atmospheres.

KNUDSON and GINSBERG (8) found no appreciable differences in the osmotic values of the saps expressed from two samples of the leaves of an unhardened greenhouse plant, *Iresine herbstii*, one frozen in an ice-salt bath, the other in liquid air. HARVEY recorded an osmotic value about 6.5 atmospheres higher for saps expressed from unhardened cabbage leaves after freezing in liquid air than for saps expressed from unhardened cabbage leaves frozen at -5° C. It is probable, however, that -5° C. is not a low enough temperature to have an effect on unhardened cabbage leaves comparable with the effect of an ice-salt bath on the leaves of *Iresine*.

The press diagrammed in text figure 1 was devised especially for the expression of leaf saps, and has been found in practice to give excellent service for this purpose. This press involves certain principles in design which apparently have not been previously employed, and has some advantages over other styles of presses in common use. The principal parts are the piston A, and the cylinder B, which were turned from steel and ground to fit within 0.001 inch. The dimensions are shown in the figure. The groove a cut around the inside wall of the cylinder one inch from the top is designed to prevent the upward movement of sap past that point. The groove b,

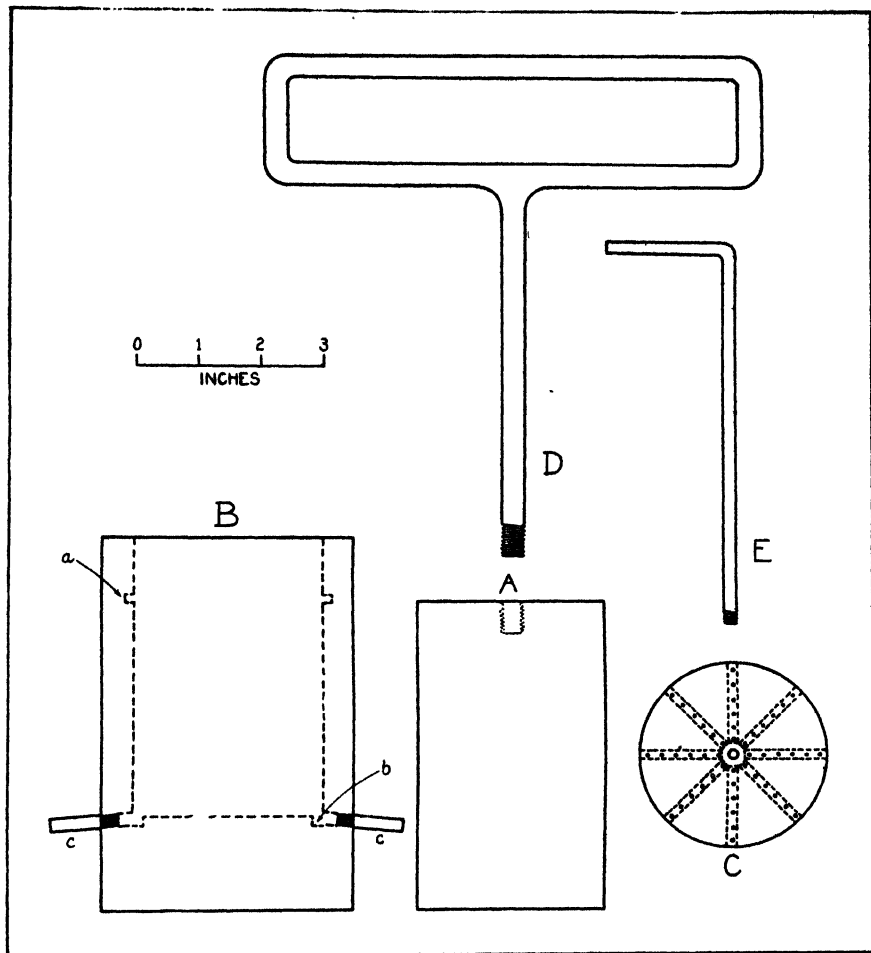


FIG. 1. Press for the expression of leaf tissue fluids. *A*, piston; *B*, cylinder; *C*, perforated disk; *D*, handle for piston; *E*, handle for disk; *a*, groove for preventing upward movement of sap; *b*, groove for collecting sap; *cc*, delivery tubes.

cut around the base of the cylinder, adjacent to the vertical wall, is designed to collect the sap and feed it to the two delivery tubes, *cc*, on opposite sides of the cylinder. These tubes are made of brass and threaded into place so they can easily be removed for cleaning. An important auxiliary part of this press is the metal disk *C*. On the lower side of this disk are eight equally spaced radiating grooves. Six one-sixteenth inch holes, spaced at equal intervals, were drilled through the metal disk into each groove. The removal of both the piston and the disk from the cylinder is facilitated by means of the metal handles, *D* and *E*, which screw into place.

By employing the metal disk described above it is possible to dispense with the use of cloth for wrapping the leaf samples. It is only necessary to place two or three disks of linen cloth, cut to fit the circumference of the cylinder snugly, on top of the metal disk in the bottom of the cylinder. The leaf sample is then placed directly on top of the cloth disks. In fact, when leaves containing considerable amounts of ligneous tissues are used, there is no need even for the disks of cloth.

This press has given satisfactory results for the expression of leaf fluids from a variety of plants. It is convenient in that it is readily handled and easily cleaned. A press of the dimensions shown in text figure 1 will accommodate leaf samples weighing up to 100 grams. Presses of this design may, of course, be built of any convenient size or, if deemed advisable, other metals than steel could be employed in their construction.

Following the suggestion of KNUDSON and GINSBERG (8) the writer has employed a materials testing machine as a source of known pressure for use with this press, with entirely satisfactory results. In packing the press it is necessary to avoid any bunching or wadding of the leaf sample. Unless the leaves are evenly distributed throughout the press cylinder, bunched portions of the sample will be subjected to a greater pressure than the more loosely packed portions. In careful work such an unequal distribution of pressure must be reduced to a minimum. It is necessary to allow the press to drain for several minutes after the maximum pressure to be employed has been reached. The most satisfactory results appear to be attained when the pressure is applied just rapidly enough to cause the expressed sap to trickle slowly from the delivery tubes.

The magnitude of the pressure used will have an effect upon the properties of the expressed sap. KNUDSON and GINSBERG (8) have shown that there is an important difference between the osmotic value of saps expressed from the leaves of *Iresine herbstii* at a pressure of 10,000 pounds and those expressed at a pressure of 50,000 pounds, both for samples frozen in liquid air and for samples frozen in an ice-salt bath. The pressure employed should depend upon the structural character of the plant organ, and upon the purpose for which the expressed fluid is to be used.

It is highly desirable that, before investigations involving determinations of the physico-chemical properties of expressed plant saps are undertaken, preliminary studies should first be conducted to determine the most suitable method of treating the tissue, and the proper magnitude of the pressure to be applied. Papers reporting the results of any such investigation should include the following information: water content of the tissue, exact treatment and method of handling the tissue, magnitude of the pressure employed, and the volume of sap expressed as a percentage of the fresh

weight or water content of the tissue. Only with such information available can the reader reconstruct for himself a reasonably accurate picture of the exact effects of the treatment and pressure upon the tissue, and only if such information be supplied can the determinations be duplicated.

Summary

1. A summary of the more important methods which have been used in the treatment of leaves before the expression of leaf saps is presented. The discussion attempts to evaluate these methods with especial regard to the limitations and advantages of each.

2. A field method of freezing leaf samples in solid carbon dioxide is described.

3. Details are given for the construction of a press which embodies certain principles in design which do not appear to have been previously used. This type of press has been found to give satisfactory results for the expression of saps from the leaves of a variety of species of plants.

LITERATURE CITED

1. ANDRÉ, G. Sur la composition des liquides qui circule dans la végétale. *Compt. Rend. Acad. Sci. (Paris)* **142**: 106–108. 1906.
2. DIXON, H. H., and ATKINS, W. R. G. Osmotic pressures in plants. I. Methods of extracting saps from plant organs. *Sci. Pro. Roy. Dub. Soc. n. s.* **13**: 422–433. 1913.
3. GAIL, F. W. Osmotic pressure of cell sap and its possible relation to winter killing and leaf fall. *Bot. Gaz.* **81**: 434–455. 1926.
4. GOLDSMITH, G. W., and SMITH, J. H. C. Some physico-chemical properties of spruce sap and their seasonal and altitudinal variation. *Colorado Coll. Pub. Sci. Ser.* **13**: 13–71. 1926.
5. GORTNER, R. A., and HARRIS, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. *Plant World* **17**: 49–53. 1914.
6. ———, LAWRENCE, J. V., and HARRIS, J. A. The extraction of sap from plant tissue by pressure. *Biochem. Bull.* **5**: 139–142. 1916.
7. HARVEY, R. B. Hardening process in plants and developments from frost injury. *Jour. Agr. Res.* **15**: 83–112. 1918.
8. KNUDSON, L., and GINSBERG, S. Suggestions with respect to the measurement of osmotic pressure. *Amer. Jour. Bot.* **8**: 164–170. 1921.

9. KORSTIAN, C. F. Density of cell sap in relation to environmental conditions in the Wasatch Mountain of Utah. *Jour. Agr. Res.* **28**: 845-907. 1924.
10. LEWIS, F. J., and TUTTLE, G. M. Osmotic properties of some plant cells at low temperatures. *Ann. Bot.* **34**: 405-416. 1920.
11. MEYER, B. S. Seasonal variations in the physical and chemical properties of pitch pine leaves, with especial reference to cold resistance. *Amer. Jour. Bot.* **15**: 449-472. 1928.
12. NEWTON, R., BROWN, W. R., and MARTIN, W. M. The extraction of plant tissue fluids and their utility in physiological studies. *Plant Physiol.* **1**: 57-65. 1926.

CHEMICAL RELATIONSHIP BETWEEN SCION AND STOCK IN CITRUS¹

A. R. C. HAAS AND F. F. HALMA

(WITH ONE FIGURE)

It is commonly known that *Citrus* varieties are propagated commercially by budding. The most important varieties grown in California are Washington Navel orange and Valencia orange (*Citrus sinensis* Osbeck), Marsh grapefruit (*Citrus maxima* Merrill), and Eureka and Lisbon lemon (*Citrus limonia* Osbeck). The rootstocks upon which these varieties are budded are sweet orange (*Citrus sinensis* Osbeck), sour orange (*Citrus aurantium* L.), grapefruit (*Citrus maxima* Merrill), and to a small extent rough lemon (*Citrus limonia*) and trifoliate orange (*Poncirus trifoliata* Raf.). The degree of congeniality shown between scion and rootstock in *Citrus* has been reported by WEBBER (7), NEWMAN (6), and others. Their observations show in a general way that the lemon scion overgrows the sour rootstock as is indicated by a bulge just above the bud union, and overgrows the sweet stock to a less extent, whereas the opposite effect is seen when the lemon is budded on grapefruit or trifoliate orange, in which case the trunk of the rootstock is larger than the lemon trunk. Similar but less pronounced size differences exist when the Navel or Valencia orange or the Marsh grapefruit is budded on sour, sweet or trifoliate rootstocks. On the other hand a smooth bud union results when these standard varieties are grown on rough-lemon rootstock. In general the degree of so-called congeniality or compatibility implies a comparison of the relative rate of growth of scion and stock; the more nearly equal the rate of growth of scion and stock the greater the congeniality or compatibility.

No adequate data are available from which to formulate a hypothesis which may lead to an understanding of the relationship of scion and stock. Limited data by JENSEN and others (5) indicate that in the case of lemon on sour stock, the amount of starch is greater above the bud union than in the case of lemon on grapefruit stock. This, however, may be considered effect rather than cause, since the overgrowth in the former case has an effect comparable to that of girdling. Our knowledge of the organic constituents of evergreen trees is scanty, and although carbohydrates may be fairly accurately determined, yet their amounts are subject to considerable fluctuation not only throughout the year but also throughout the day. On the other hand the inorganic constituents of mature parts of *Citrus* trees

¹ Paper no. 205, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

fluctuate very little and hence studies along this line are more promising. For this reason we have studied the inorganic composition of scion and stock in Citrus.

Before reporting the results of the present investigation it is necessary to refer to previous work which shows that fundamental physical and chemical differences exist in the leaves of varieties of Citrus that are ordinarily combined in budding. The ash and calcium of the water-soluble fraction of mature normal Washington Navel and Valencia orange leaves exceed those of Eureka lemon (HAAS, 1). The sap of normal mature lemon leaves is less active osmotically and contains less ash and calcium but more magnesium than the sap of orange leaves (HAAS and HALMA, 2). Investigations on the sap and the morphology of leaves of these two Citrus species indicate that the lemon leaf has a more efficient photosynthetic system than the orange leaf (HALMA and HAAS, 3; HALMA, 4). Furthermore, it is a matter of common observation that the growth rate of unbudded seedlings or cuttings of lemon is greater than that of sour orange, sweet orange or grapefruit, but about equal to that of rough lemon. It seems reasonable to assume that varieties or species that differ in so many respects must undergo some changes in their physical or chemical make-up when combined with one another by means of budding. The data presented herewith show that a chemical difference exists between scion and stock. It appears that the scion species or variety influences the amount of soluble magnesium in the bark of the stock.

The material analyzed consisted entirely of bark. In all cases the cambium was active so that it was an easy matter to remove the bark. Care was taken to collect material only from trees growing on definitely known stocks. The bark was obtained from areas immediately above and below the bud union. The portion of the tree from which the material was taken was carefully cleaned; the bark was peeled off and dried in an oven at 65° C. The dried material was finely pulverized so as to pass through a 20-mesh sieve. Water-soluble and insoluble ash, calcium and magnesium were determined. The analytical procedure was essentially the same as that previously used (1).

Previous studies (1) have shown that the leaves of sweet orange and lemon could be distinguished by the water solubility of the calcium in the dried leaves: the dry matter of the sweet orange leaves contained about two and one-half times as much water-soluble calcium as that of the lemon leaves. It was assumed in the present investigation that possibly a similar chemical difference may exist in the bark. It was further assumed that, if this were found to be the case, it might serve as a means for identifying the variety by means of bark analysis.

From table I, however, it is clear that this calcium relationship does not exist in the bark, where the water solubility is extremely low as compared with that of the leaves. It may be of interest to add that the total calcium as a percentage of the dry matter is about equal to that found in the leaves.

Table I suggests that the amount of soluble magnesium as a percentage of the dry matter or of soluble ash depends upon the position which the variety occupies in the budded tree; for where the sweet orange served as the scion, its soluble magnesium content was considerably higher than when it served as a stock for the lemon scion. It is also clear from table I that sour, sweet and grapefruit stocks gave values similar to that of the lemon scion.

Since the material in table I was considered inadequate to definitely establish this magnesium relationship between scion and stock, a sufficiently large number of bark samples representing various commonly occurring combinations of scion and stock were collected. These samples were obtained from trees varying in age from seven to thirty-five years and growing under widely different soil and climatic conditions in southern California. A limited number of samples were also obtained from unbudded trees. Because of the possible influence of seasonal variations, the material was collected both in March and in August of the same year. These samples were analyzed by the same procedure as was used for the previous material.

The data given in table II are so grouped that the magnesium relationship between scion and stock of the same tree may very readily be seen. Figure 1 gives the average values and enables one to summarize the data readily. If we consider the trees having a lemon scion we find that the average values for the soluble magnesium as a percentage of the dry matter are rather uniform both in the scion and stock, regardless of the four varieties of stock used. On the other hand if the scion is Valencia orange or Marsh grapefruit the values for the soluble magnesium are considerably higher, not only in the scion but also in the stock, which in this case includes both sweet and sour orange. The same type of sour orange which served as a stock for the lemon scion shows a higher soluble magnesium content when, instead of a lemon scion, it had a Marsh grapefruit or Valencia orange scion.

Turning to the unbudded trees where there is no scion influence, we find that the bark of sour orange and rough lemon is appreciably lower in soluble magnesium than that of either sweet orange or grapefruit. The values for unbudded sour and rough-lemon seedlings are not changed appreciably by budding a lemon scion on them. However, the value for

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES. (MATERIAL COLLECTED OCTOBER, 1927)

SAMPLE NO.	MATERIAL	TOTAL ASH IN DRY MATTER	SOLUBLE ASH IN		TOTAL CALCIUM IN		SOLUBLE CALCIUM IN		TOTAL MAGNESIUM IN		SOLUBLE MAGNESIUM IN			
			TOTAL ASH	DRY MATTER	TOTAL ASH	DRY MATTER	SOLUBLE ASH	DRY MATTER	TOTAL ASH	DRY MATTER	SOLUBLE ASH	DRY MATTER		
17	{ Eureka lemon scion (Sour stock)	14.38	9.23	1.33	36.45	5.24	0.12	6.13	1.55	2.02	0.29	0.048	3.60	16.46
18	{ Sour stock of above tree	14.88	7.47	1.11	36.85	5.48	0.08	7.43	1.51	0.75	0.11	0.051	4.61	46.00
19	{ Eureka lemon scion													
20	{ (Sour stock)	14.69	5.81	0.85	37.96	5.58	0.11	12.32	1.89	1.31	0.19	0.038	4.41	19.54
21	{ Sour stock of above tree	15.34	6.99	1.07	37.61	5.77	0.09	8.45	1.57	1.31	0.20	0.044	4.15	22.13
	{ Eureka lemon scion													
22	{ (Sour stock)	14.89	8.08	1.20	36.77	4.52	0.09	7.59	2.01	1.44	0.18	0.035	3.13	21.13
2	{ Sour stock of above tree	12.36	9.69	1.20	36.56	5.48	0.09	7.74	1.70	1.22	0.18	0.038	2.94	19.38
	{ Sour stock (Eureka lemon scion)													
3	{ " " " "	12.77	10.51	1.34	35.65	4.55	0.13	9.49	2.80	1.29	0.16	0.054	4.01	32.76
6	{ " " " "	13.48	8.89	1.20	35.48	4.78	0.13	10.57	2.65	1.04	0.14	0.051	4.29	36.59
7	{ " " " "	12.56	9.75	1.23	35.69	4.48	0.14	11.09	3.03	0.90	0.11	0.041	3.35	36.36
7	{ " " " "	13.58	8.51	1.16	35.60	4.83	0.11	9.40	2.25	0.94	0.13	0.045	3.89	35.42
8	{ " " " "	13.66	8.83	1.21	35.45	4.84	0.14	11.97	2.98	0.70	0.10	0.037	3.05	38.24
15	{ " " " "	10.75	6.08	0.94	36.77	5.72	0.10	10.91	1.80	0.66	0.10	0.031	3.30	30.23
15	{ " " " "	7.93	8.41	1.20	37.09	5.27	0.24	19.85	4.50	1.00	0.14	0.053	4.39	36.84
23	{ Sweet " " " "	18.31	7.29	1.33	32.82	6.01	0.16	11.73	2.61	0.70	0.13	0.043	3.18	35.19
14	{ " " " "	16.56	9.22	1.53	36.02	5.97	0.21	13.57	3.47	0.72	0.12	0.058	3.78	48.21
12	{ Grapefruit stock (Eureka lemon scion)													
16	{ " " " "	18.81	8.66	1.63	30.10	5.66	0.17	10.57	3.04	1.00	0.19	0.057	3.52	30.38
16	{ " " " "	17.15	7.28	1.25	35.58	6.10	0.12	9.67	1.98	0.72	0.12	0.032	2.60	26.09
1	{ Valencia scion (Sweet stock)	13.70	10.31	1.41	35.62	4.88	0.19	13.66	3.95	1.36	0.19	0.072	5.12	38.89
5	{ " " " "	13.31	11.79	1.57	35.20	4.68	0.24	15.28	5.12	1.39	0.18	0.095	6.08	51.56
10	{ " " " "	12.94	11.49	1.48	35.91	4.65	0.22	14.58	4.67	1.03	0.13	0.084	5.66	63.46
4	{ " " " "	11.02	13.12	1.45	34.67	3.82	0.16	11.23	4.60	1.95	0.21	0.084	5.81	39.19
9	{ " " " "	10.54	12.75	1.34	34.15	3.60	0.15	11.21	4.19	1.76	0.19	0.073	5.42	39.19
11	{ " " " "	10.86	12.12	1.31	34.52	3.75	0.14	11.01	3.86	1.61	0.17	0.071	5.39	40.68
24	{ " " " "	6.77	10.18	1.50	34.06	5.03	0.22	14.69	4.39	1.69	0.24	0.100	6.78	42.86

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES

SAMPLE NO.	DATE COLLECTED 1928	MATERIAL	TOTAL ASH IN			SOLUBLE ASH IN			TOTAL CALCIUM IN			SOLUBLE CALCIUM IN			TOTAL MAGNESIUM IN			SOLUBLE MAGNESIUM IN		
			TOTAL ASH	DRY MAT-TER	ASH	TOTAL ASH	DRY MAT-TER	ASH	TOTAL ASH	DRY MAT-TER	ASH	TOTAL ASH	DRY MAT-TER	ASH	TOTAL ASH	DRY MAT-TER	ASH	TOTAL ASH	DRY MAT-TER	ASH
9	March	{ Eureka lemon scion (sour stock) }	11.51	10.29	1.18	35.82	4.12	0.11	9.29	2.67	0.36	0.04	0.020	1.69	47.62	1.69	47.62			
10	"	{ Sour stock of above tree }	12.65	7.97	1.01	37.79	4.78	0.13	13.29	2.80	0.68	0.09	0.034	3.37	39.53	3.37	39.53			
12	"	{ Lisbon lemon scion (sour stock) }	13.41	9.41	1.43	35.60	4.78	0.09	6.31	1.88	0.75	0.10	0.032	2.24	32.00	2.24	32.00			
13	"	{ Sour stock of above tree }	13.51	9.67	1.31	36.33	4.91	0.13	10.00	2.66	0.57	0.08	0.032	2.45	41.38	2.45	41.38			
5	"	{ Sour seedling (unbudded) }	13.43	9.78	1.30	34.94	4.69	0.10	7.48	2.07	0.70	0.09	0.024	2.49	34.29	2.49	34.29			
12	August	" " " " " "	12.04	10.08	1.21	37.94	4.57	0.09	7.08	1.98	0.95	0.11	0.020	1.05	17.54	1.05	17.54			
13	"	" " " " " "	15.09	5.38	0.81	39.12	5.90	0.10	12.07	1.66	0.78	0.12	0.034	4.19	28.81	4.19	28.81			
18	March	{ Eureka lemon scion (rough lemon stock) }	12.35	8.45	1.04	36.52	4.51	0.08	7.28	1.69	0.52	0.06	0.020	1.72	28.12	1.72	28.12			
19	"	{ Rough lemon stock of above tree }	12.26	8.16	1.52	36.26	6.76	0.11	6.92	1.56	0.40	0.07	0.026	1.73	35.71	1.73	35.71			
22	"	{ Eureka lemon scion (rough lemon stock) }	11.97	8.99	1.08	36.49	4.37	0.08	7.43	1.83	0.62	0.07	0.022	2.04	29.73	2.04	29.73			
23	"	{ Rough lemon stock of above tree }	19.21	8.61	1.65	36.14	6.94	0.11	6.47	1.54	0.40	0.08	0.039	2.38	51.85	2.38	51.85			
11	August	{ Rough lemon seedling (unbudded) }	16.85	8.49	1.43	39.01	6.57	0.10	6.85	1.49	0.54	0.09	0.034	2.38	37.78	2.38	37.78			
48	"	" " " " " "	13.11	9.90	1.30	37.49	4.92	0.12	8.94	2.36	0.99	0.13	0.038	2.93	29.23	2.93	29.23			
6	March	{ Eureka lemon scion (sweet stock) }	12.42	10.56	1.31	34.50	4.28	0.08	6.41	1.96	0.66	0.08	0.030	2.25	36.11	2.25	36.11			
7	"	{ Sweet stock of above tree }	15.45	9.77	1.51	34.36	5.31	0.15	9.80	2.79	1.05	0.16	0.044	2.91	27.16	2.91	27.16			
32	"	{ Eureka lemon scion (sweet stock) }	11.90	11.36	1.35	34.29	4.08	0.07	5.34	1.77	0.59	0.07	0.021	1.55	30.00	1.55	30.00			
33	"	{ Sweet stock of above tree }	14.58	9.00	1.31	35.67	5.20	0.14	10.82	2.73	0.25	0.04	0.032	2.44	88.89	2.44	88.89			
26	"	{ Lisbon lemon scion (sweet stock) }	15.71	5.16	0.81	37.43	5.88	0.09	11.36	1.56	0.55	0.09	0.024	2.96	27.91	2.96	27.91			
27	"	{ Sweet stock of above tree }	16.19	9.52	1.54	37.12	6.01	0.20	12.82	3.29	0.81	0.13	0.059	3.82	44.94	3.82	44.94			
28	"	{ Lisbon lemon scion (sweet stock) }	15.84	5.27	0.84	38.31	6.07	0.09	10.04	1.46	0.49	0.08	0.027	3.19	34.29	3.19	34.29			
7	August	{ Sweet seedling (unbudded) }	16.60	7.71	1.28	38.82	6.45	0.12	12.66	2.51	1.30	0.22	0.070	5.47	32.40	5.47	32.40			
47	"	" " " " " "	15.36	9.06	1.39	37.76	5.80	0.17	12.36	2.97	1.34	0.21	0.070	5.03	33.98	5.03	33.98			
52	"	" " " " " "	15.64	8.30	1.30	38.99	6.10	0.19	14.33	3.05	1.33	0.21	0.070	5.39	33.65	5.39	33.65			
20	March	{ Lisbon lemon scion (grapefruit stock) }	12.13	11.13	1.21	35.87	4.35	0.11	8.15	2.35	0.41	0.05	0.022	1.63	44.00	1.63	44.00			
21	"	{ Grapefruit stock of above tree }	15.10	11.18	1.70	35.88	5.45	0.19	11.43	3.56	0.54	0.08	0.034	2.00	41.46	2.00	41.46			
24	"	{ Lisbon lemon scion (grapefruit stock) }	10.10	13.88	1.40	34.03	3.44	0.08	6.00	2.45	0.95	0.09	0.033	2.33	34.14	2.33	34.14			
25	"	{ Grapefruit stock of above tree }	13.09	13.88	1.82	35.03	4.58	0.21	11.55	4.57	1.06	0.14	0.064	3.03	39.53	3.03	39.53			

TABLE II—(Concluded)

INORGANIC COMPOSITION OF BARK OF DIFFERENT VARIETIES OF CITRUS SCIONS AND STOCKS EXPRESSED AS PERCENTAGES

SAMPLE NO.	DATE COLLECTED 1928	MATERIAL	TOTAL ASH IN			TOTAL CALCIUM IN			SOLUBLE CALCIUM IN			TOTAL MAGNESIUM IN			SOLUBLE MAGNESIUM IN		
			TOTAL	DRY MATTER	ASH	TOTAL	DRY MATTER	ASH	DRY MATTER	SOLUBLE	CALCIUM	TOTAL	DRY MATTER	ASH	DRY MATTER	SOLUBLE	MAGNESIUM
30	March	{ Lisbon lemon scion (grapefruit stock).	17.73		4.35	0.77	6.60	37.22	0.07	9.85	1.15	0.62	0.11	0.020	2.59	18.16	
31	"	{ Grapefruit stock of above tree.....	15.31		6.65	1.02	5.71	37.30	0.13	12.78	2.28	0.63	0.10	0.030	2.91	30.95	
34	"	{ Eureka lemon scion (grapefruit stock).	9.37		10.21	0.96	3.30	35.16	0.10	10.02	2.91	1.28	0.12	0.031	3.20	25.42	
35	"	{ Grapefruit stock of above tree.....	12.02		9.68	1.16	36.90	4.44	0.14	11.86	3.11	0.88	0.11	0.038	3.26	36.85	
14	August	{ Grapefruit seedling (unbudded).....	17.05		9.16	1.56	6.51	38.17	0.22	14.21	3.41	1.43	0.24	0.086	5.51	35.26	
17	"	{ Eureka lemon scion.....	10.99		9.92	1.09	38.44	4.22	0.13	10.28	2.65	1.29	0.14	0.042	3.85	29.58	
4	"	{ Marsh grapefruit intermediate.....	13.37		8.52	1.14	35.44	4.74	0.13	11.58	2.78	0.73	0.10	0.032	2.81	32.65	
8	"	{ Sweet stock	15.56		8.24	1.28	36.21	6.10	0.13	9.83	2.07	0.78	0.12	0.038	2.96	31.15	
20	"	{ Eureka lemon scion.....	11.47		10.72	1.23	36.17	4.15	0.10	8.29	2.46	1.22	0.14	0.032	2.80	26.67	
18	"	{ Marsh grapefruit intermediate.....	13.92		8.92	1.24	35.05	5.30	0.13	10.47	2.45	0.93	0.13	0.036	2.90	26.87	
1	"	{ Sweet stock	16.17		8.53	1.38	39.14	6.33	0.14	10.00	2.18	0.79	0.13	0.036	2.61	28.12	
53	"	{ Eureka lemon scion.....	10.18		10.96	1.12	37.65	3.83	0.11	10.22	2.97	1.36	0.14	0.030	2.69	21.74	
9	"	{ Marsh grapefruit intermediate.....	13.50		8.83	1.19	37.74	5.10	0.11	9.06	2.12	0.95	0.13	0.030	2.52	23.44	
2	"	{ Sweet stock	15.89		8.21	1.30	38.89	6.18	0.12	8.90	1.88	0.73	0.12	0.036	2.76	31.03	
16	"	{ Marsh grapefruit scion (sweet stock)...	14.26		9.13	1.30	38.34	5.47	0.14	10.91	2.60	1.51	0.22	0.070	5.38	32.40	
15	"	{ Sweet stock of above tree.....	16.07		9.07	1.46	38.72	6.20	0.16	10.29	2.41	1.07	0.17	0.054	3.70	31.40	
19	"	{ Marsh grapefruit scion (sweet stock)...	14.09		9.35	1.32	37.28	5.25	0.16	12.29	3.08	1.60	0.25	0.060	4.55	26.55	
22	"	{ Sweet stock of above tree.....	15.42		8.67	1.34	37.67	5.81	0.17	13.00	2.99	1.15	0.18	0.066	4.93	37.08	
6	"	{ Marsh grapefruit scion (sweet stock)...	14.25		9.39	1.34	38.60	5.50	0.19	14.50	3.53	1.80	0.26	0.094	7.03	36.72	
51	"	{ Sweet stock of above tree.....	16.43		8.40	1.38	40.16	6.60	0.15	10.87	2.27	1.14	0.19	0.062	4.49	32.98	
3	March	{ Marsh grapefruit scion (sour stock)...	12.94		14.04	1.82	33.69	4.36	0.19	10.46	4.36	1.61	0.21	0.089	4.85	42.31	
4	"	{ Sour stock of above tree.....	11.14		12.59	1.40	34.12	3.80	0.15	10.41	3.84	1.72	0.19	0.068	4.85	35.42	
14	"	{ Marsh grapefruit scion (sour stock)...	12.68		14.99	1.90	33.79	4.28	0.21	10.84	4.81	1.28	0.18	0.086	4.53	53.09	
15	"	{ Sour stock of above tree.....	11.56		12.15	1.40	35.50	4.10	0.13	9.26	3.17	1.44	0.17	0.067	4.70	39.76	
1	"	{ Valencia orange scion (sour stock)....	12.39		15.43	1.91	33.08	4.10	0.23	12.03	5.61	1.69	0.21	0.110	5.75	52.38	
2	"	{ Sour stock of above tree.....	10.05		13.57	1.34	34.17	3.43	0.16	11.58	4.60	1.37	0.14	0.066	4.11	40.58	
16	"	{ Valencia orange scion (sour stock)....	12.01		18.39	2.21	33.32	4.00	0.24	11.05	6.10	2.39	0.29	0.140	6.10	47.22	
17	"	{ Sour stock of above tree.....	10.22		15.90	1.63	33.90	3.47	0.16	9.96	4.67	2.27	0.23	0.104	6.40	44.83	

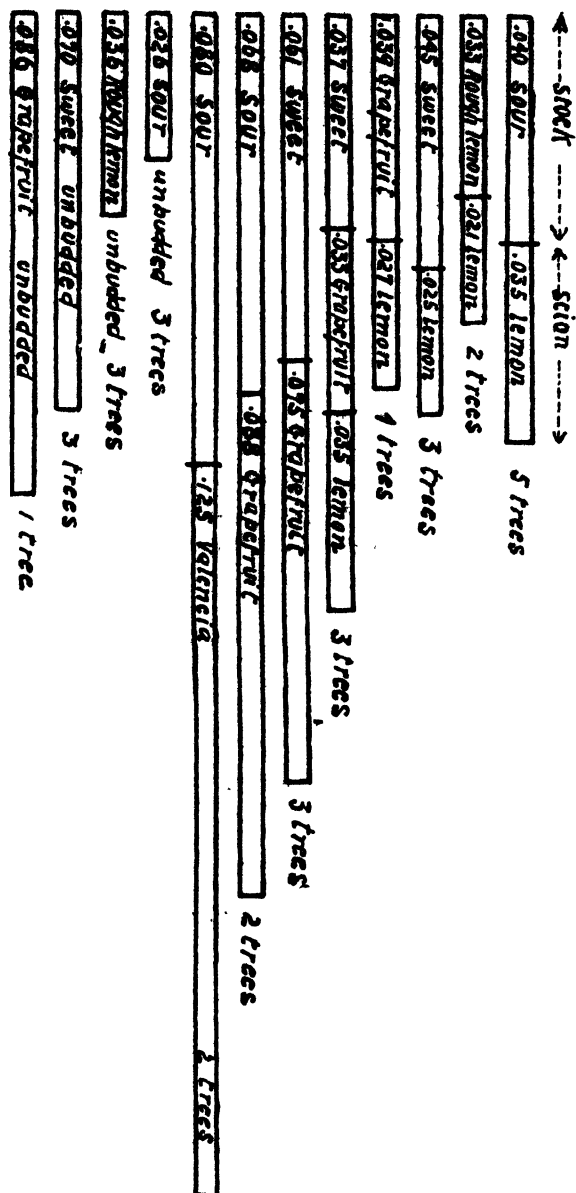


FIG. 1. Average values for soluble magnesium as a percentage of dry matter for stock and scion.

sour orange when used as a stock for either Valencia orange or Marsh grapefruit scions, is increased above the value found for unbudded sour seedlings.

A clear case of the dominance exerted by the scion as far as soluble magnesium is concerned is seen in trees which were originally Marsh grapefruit on sweet stock, but which were later topworked to Eureka lemon. In this case the values for the grapefruit intermediate as well as for the sweet stock are approximately the same as those for the lemon scion. Other trees of the same age growing in the same orchard, which were not topworked, and hence retained their grapefruit top, showed values for both scion and stock which were considerably higher than those found in topworked trees. In other words the replacement of the original grapefruit top by the lemon scion exerted a profound influence on both the original scion and the stock. Therefore, as far as soluble magnesium content is concerned, there seems to be no very marked stock influence upon the lemon scion. On the other hand, as is seen in figure 1, when the grapefruit or sweet orange scion is on sour stock, which when unbudded has a low soluble magnesium content, the value for the stock is greatly increased. But we also see that the unbudded sweet orange and grapefruit have higher values than the unbudded sour orange. Here again we cannot escape the conclusion that the grapefruit or Valencia scion was responsible for the increase in the values found in the sour stock.

It appears from figure 1 that there may be an increase in soluble magnesium as one passes from the lemon scion to the various stocks, but the reverse is true to an even greater extent when the scion is Valencia or grapefruit. The reasons for the influence of scion on stock are unknown but it may be suggested that the differences in the growth rate between lemon and sweet orange or grapefruit may play an important rôle in that the leaves of the former would draw more heavily on the magnesium supply than those of the latter.

There are several points of interest in the tables in addition to the case of soluble magnesium. It is well known that the calcium content of the ash of normal mature Citrus leaves is nearly constant, being about 35 per cent. Approximately the same percentage was found in the ash of all bark samples analyzed, regardless of whether the variety was the scion or the stock. Moreover the dry matter of the lemon leaves contains about 18 per cent. of soluble calcium, whereas that of sweet orange contains approximately 50 per cent. (1). Such differences do not exist in the trunk bark; as a matter of fact, the values as given in the tables are extremely low in comparison and do not reveal any great difference between the different varieties.

Throughout the tables there is a slight suggestion of inorganic gradients in the trunk. Since the samples consisted of bark taken immediately above and below the bud union, one could not expect to find consistent differences

in the same direction. However, in the case of the trees having a lemon scion, a grapefruit intermediate and a sweet stock, the samples necessarily represent greater intervals along the trunk and main branches. Here we find a gradual increase from top to bottom in the soluble and total calcium in the dry matter, but a decrease in the soluble ash and total magnesium in the total ash.

Summary

Some differences in inorganic composition between scion and stock of Citrus were studied. It was found that the soluble magnesium as a percentage either of the dry matter or of the soluble ash of the trunk bark is lowest in the lemon and sour orange and highest in the sweet orange and grapefruit. The values found in the bark of the stock vary according to the scion variety with which it is combined. If the scion is lemon, which normally has a low value, and the stock is sweet orange or grapefruit, the values for the stocks are much lower than they would be if they had not been budded. On the other hand if sweet orange or grapefruit (with a higher value than the lemon) is growing on the sour orange, then the value of the latter is considerably higher than it would be if the lemon were the scion.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. HAAS, A. R. C. Water-solubility of dry matter in relation to calcium nutrition of normal orange and lemon leaves. *Bot. Gaz.* **85**: 334-340. 1928.
2. ———, and HALMA, F. F. Physical and chemical characteristics of expressed Citrus leaf sap and their significance. *Bot. Gaz.* **85**: 457-461. 1928.
3. HALMA, F. F., and HAAS, A. R. C. Effect of sunlight on sap concentration of Citrus leaves. *Bot. Gaz.* **86**: 102-106. 1928.
4. ———. Quantitative differences in palisade tissue in Citrus leaves. *Bot. Gaz.* **87**: 319-324. 1929.
5. JENSEN, C. A., WILCOX, L. V., and FOOT, F. J. Behavior of different lemon bud and rootstock combinations at Limoneira. *California Citrograph* **12**: 234, 266, 267, 268. 1927.
6. NEWMAN, C. V. Responses of Citrus trees to different rootstocks. *California Citrograph* **11**: 375, 406, 407. 1926.
7. WEBBER, H. J. Rootstock reactions as indicating the degree of congeniality. *Proc. Amer. Soc. Hort. Sci.* 1926: 30-36.

STORAGE OF TRUCK CROPS: THE GIRASOLE, *HELIANTHUS TUBEROSUS*¹

HAMILTON P. TRAUB,² CLIFFORD J. THOR, J. J. WILLAMAN,
AND E. OLIVER

(WITH FOUR FIGURES)

The girasole,³ *Helianthus tuberosus*, has had much interest centered upon it since the report of JACKSON, SILSBEE and PROFFITT (4) on the possible commercial preparation from it of fructose, a sugar sweeter than sucrose, according to PAUL (7), DEERE (3), SALE and SKINNER (8), BIESTER, WOOD and WAHLIN (2) and SPENGLER and TRAEGER (10). A survey of the plants which might serve as a source for the commercial production of fructose shows that the girasole ranks among the more promising. The plant has been in general use in many European countries for a long time as a truck crop and as a stock food, and to a lesser extent this is true for certain sections of the United States.

One of the chief problems in connection with this crop is that of storage. The periderm of the girasole tuber is very thin and easily ruptured. There is a rapid loss of moisture on exposure to atmosphere at room temperature. The tubers are subject to parasitic diseases under ordinary storage conditions.⁴ Most authorities seem to be agreed that the best method of storage is the practice of leaving the tubers in the ground and digging them when needed. SHOEMAKER (9) reports good success with tubers stored in burlap bags in a cold cellar at Washington, D. C., from November, 1925, to March, 1926, without shriveling or other difficulty. The temperature was kept near the freezing point. The preliminary experiments reported in this paper covering the period from August 30, 1927, to March 22, 1928, were carried out in order to secure more accurate information with regard to the storage of this crop.

* Published, with the approval of the Director, as paper no. 807 of the Journal Series of the Minnesota Agricultural Experiment Station.

¹ Thanks are due LAWRENCE ZELENY for competent assistance in certain of the chemical determinations.

² Chief, Division of Horticulture, Texas Agricultural Experiment Station, College Station, Texas, since April, 1928.

³ *Helianthus tuberosus* is not an artichoke and is not native to Palestine; therefore, "Jerusalem artichoke" is a lengthy misnomer, and is being replaced, for purposes of economy, if for no other reason, by the simpler term, "girasole." See L. H. BAILEY, Vegetable Gardening, 1890.

⁴ A study of the diseases of girasole tubers in storage was carried on parallel with the experiments reported in this paper by Prof. H. W. JOHNSON, of the Division of Plant Pathology. This report will be published separately.

Methods

The general plan of the experiment included the study of the dry matter and water soluble carbohydrates in the tubers of the Portland variety from August 30, 1927 to March 22, 1928, (1) outdoors on silt loam, (2) outdoors on sandy loam, (3) in common storage at 34°–49.5° F., (4) common storage at 33.5°–44° F., (5) in cold storage at 32°–35° F. The tubers were washed, dried with a towel and ground to a pulp in a food chopper. The pulp was thoroughly mixed, after which 100 gm. were taken for a moisture determination, and two 30-gm. portions for sugar analysis. Fifteen cc. of water were added to each of the 30-gm. samples in a 250-cc. beaker and the mixture was set in a boiling water bath for one hour. The extract was pressed from the pulp while hot, with a small juice press, into a 100-cc. volumetric flask. The pulp was washed with two 15-cc. portions of boiling water, the washings being added to the first extract. The combined extracts were hydrolyzed with 2.5 cc. of 8.12 N HCl on a water bath at 70–80° C., for 35 minutes and cooled to room temperature. To clarify the hydrolyzed extract, solid neutral lead acetate was added until precipitation was complete. A white precipitate of lead chloride is deposited first and this is followed by colloidal matter. The mixture was then diluted to volume (100 cc.) and filtered, delead with solid disodium phosphate and filtered again.

Aliquots of the delead filtrate, which was clear and practically colorless, were used for the selective determination of fructose and total reducing substances by Osr's cupro-carbonate method as modified by Nyns (5). The cupro-carbonate solution was prepared as follows: 250 gm. K_2CO_3 and 100 gm. $KHCO_3$ were dissolved in 700 cc. boiling water; 25.3 gm. of very pure $CuSO_4 \cdot 5H_2O$ dissolved in about 100 cc. of water were added, and the solution was made up to 1 liter. One or 2-cc. aliquots of the clarified sugar solution were placed in 250-cc. Erlenmeyer flasks and distilled water was added to make a total of 20 cc. Then 50 cc. of cupro-carbonate solution were added and the mixture was reduced: one set on a water bath kept at 48.5–49° C. for the determination of fructose, and another set on a boiling water bath for the determination of total water soluble carbohydrates. Reduction was carried out for exactly 2.5 hours, when the cuprous oxide precipitates were collected in Gooch crucibles on asbestos mats. The amount of copper was determined in each case by the permanganate method as given in Official Methods (1). These values, referred to tables in OLIVER's paper (6), gave the amount of fructose and of total reducing sugars. Subtracting the former from the latter gave "glucose," a term here used to include glucose and any other reducing substances other than fructose which may be present.

Kinds of carbohydrates present

In an attempt to determine the amount of free reducing sugars in the girasole tuber, a sample (Nov. 3, 1927) was extracted in the usual way, except that some CaCO_3 was added to prevent hydrolysis of polysaccharides by the natural acidity of the juice. Hydrolysis with HCl was omitted but the extract was clarified with neutral lead acetate and delead as usual. This unhydrolyzed extract was subjected to the cupro-carbonate reduction at $48.5\text{--}49^\circ \text{C.}$, and at 100°C. In both cases no more copper was reduced than in the case of the blank for the method, indicating the absence of free fructose or free "glucose."

Samples of the same material were extracted at room temperature (23°C.) instead of at 100° and in the presence of CaCO_3 to prevent hydrolysis. In this case the extract was also filtered to remove CaCO_3 particles. The extract was then hydrolyzed with HCl and run through the usual procedure. In this way it was hoped to remove the water soluble levulins or inulides without removing inulin itself. However, the analyses gave only slightly lower values for fructose and "glucose" in this extract than in the case of the extracts prepared in the usual way.

	Fructose per cent.	"Glucose" per cent.
Usual method	10.90	5.71
Cold extraction in presence of CaCO_3 and hydrolysis with HCl	9.68	4.97

This indicates that practically all the water soluble polysaccharides are inulides, and that the inulin content is low, which is in harmony with the work of WILLAMAN (12).

Preliminary analyses, 1926-27

The preliminary work was done on the crop of 1926. One variety of white girasole was grown in St. Louis County. Another lot of tubers, of an unknown variety, was secured from Portland, Oregon, and grown at University Farm; and some of the same variety, which had been grown at Portland, were sent to us in October and placed in a root cellar. Some French Mammoth were also grown at University Farm. Samples of tubers were dug at intervals during the winter and spring, some being analyzed immediately and others kept in a root cellar for various periods. The data are presented in table I.

It will be noted that the fructose content is rather low in comparison with that found by JACKSON, SILSBEE and PROFFITT (4). This is probably due to immaturity. The sample from Portland, Oregon, contains nearly twice as much fructose as the others.

During the winter, whether the tubers are in the ground or in storage, the fructose-glucose ratio is rather low. By May the ratio has increased

TABLE I
COMPOSITION OF GIRAOLE TUBERS, CROP OF 1926

DATE DUG	STORAGE	FRUCTOSE		GLUCOSE*		FRUCTOSE PLUS GLUCOSE		FRUCTOSE GLUCOSE
		IN TUBERS	IN EXTRACT- ABLE DRY MATTER	IN TUBERS	IN EXTRACT- ABLE DRY MATTER	IN TUBERS	IN EXTRACT- ABLE DRY MATTER	
October " " " " " " " " " "	10 weeks in root cellar	per cent.	per cent.	Grown in St. Louis County, Minnesota				
	11 weeks in root cellar	10.08	3.72	per cent.				
	12 weeks in root cellar	7.66	4.75	13.80				
	13 weeks in root cellar	8.42	4.78	12.41				
	15 weeks in root cellar	8.72	5.14	13.20				
	16 weeks in root cellar	8.24	4.56	13.86				
October February 23 March 12 " 12 " 30 April 9 " 18 " 29 May 25	5 months in sand in root cellar	Variety grown at Portland, Oregon		per cent.				
		15.47	8.28	23.75				
	freshly dug	Variety from Portland, Oregon, grown at University Farm		per cent.				
	freshly dug	7.91	5.07	12.98				
	6 weeks in root cellar	7.88	5.30	13.18				
	3 weeks in root cellar	7.20	41.8	12.14				
April 9 " 18 " 29 May 25	freshly dug	7.06	5.66	12.72				
	freshly dug	7.56	5.09	12.65				
	freshly dug	5.64	35.2	33.6				
	freshly dug	6.34	32.5	11.02				
	freshly dug	6.23	39.2	9.66				
		French Mammoth, grown at University Farm		17.0				
February 23 March 14 " 30 April 9 " 9 May 10 " 25	1 week in root cellar	10.30	4.37	8.03				
	1 week in root cellar	8.02	4.27	14.67				
	freshly dug	8.45	4.49	12.29				
	freshly dug	7.84	5.50	12.94				
	4 weeks in root cellar	7.23	42.4	13.34				
	freshly dug	7.23	4.87	12.10				
	freshly dug	6.73	2.86	71.0				
	freshly dug	6.33	41.9	55.3				
		French Mammoth, grown at University Farm		16.5				
		10.30	4.37	55.1				

* Glucose here means reducing sugars other than fructose in the hydrolyzed extract.

markedly. It must be pointed out that direct comparison may not be made between these 1926 samples and those grown in 1927.

Experiments in 1927-28

The Portland variety of girasole was grown at University Farm in 1927 in sandy loam and in silt loam. They were analyzed when freshly dug on August 30, when the tubers were still immature; on November 3, when they had apparently gained their maximum development; and on December 20, January 30, and March 22, during the storage period. On November 3, some of the tubers on the sandy loam were dug and placed in three types of storage. The conditions of storage and the behavior of the tubers under these conditions is shown in table II and figure 1. The results of the analyses are shown in table III and in figures 2, 3, and 4.

TABLE II

PER CENT. OF SOUND, SHRIVELED AND DISEASED GIRASOLE TUBERS, UNDER DIFFERENT STORAGE CONDITIONS. GROWN AT UNIVERSITY FARM, ST. PAUL, MINNESOTA

STORAGE CONDITIONS		DATE OF INSPECTION	CONDITION OF TUBERS EXPRESSED IN PER CENT. OF ORIGINAL NUMBERS		
TEMPERATURE	RELATIVE HUMIDITY		SOUND	SHRIVELED	DISEASED
	<i>per cent.</i>		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Common storage 34-49.5° F.	73.9-86.1	January 30, 1928	27.7	38.7**	33.5
		March 29, 1928	7.5	6.9**	85.5
Common storage 33.5-44° F.	89.3-92.0	January 30, 1928	48.6	33.3**	18.0
		March 29, 1928	47.3	44.6**	19.3
Cold storage 32-35° F.	89.1-92.0	January 30, 1928	85.2	10.0*	4.7
		March 29, 1928	80.5	14.1*	5.2

* Slightly shriveled.

** Much shriveled.

Condition of tubers.—The data in the table indicate that the higher temperatures and lower humidities induce the shrivelling of the tubers as well as the attack by pathogenic organisms. On the other hand, the tubers keep very well at lower temperatures and higher humidities. Under the most favorable conditions supplied in these experiments (32°-35° F., and 89-92 per cent. humidity) 80 per cent. of the tubers were sound at the end of the period, less than 15 per cent. were slightly shrivelled, and 5 per cent. were diseased. Under the least favorable conditions (34°-49° F. and 74-86 per cent. humidity) only 7 per cent. remained sound, 6 per cent. were shrivelled and 85 per cent. were diseased.

TABLE III

SEASONAL VARIATION IN MOISTURE, DRY MATTER, FRUCTOSE AND TOTAL WATER SOLUBLE CARBOHYDRATES IN GIRASOLE TUBERS, PORTLAND VARIETY, IN THE GROUND AND IN STORAGE. GROWN AT UNIVERSITY FARM, ST. PAUL, MINNESOTA

STORAGE CONDITIONS	GREEN WEIGHT BASIS					DRY WEIGHT BASIS			RATIOS		
	DATE	MOISTURE	DEY MATTER	FRUCTOSE	GLUCOSE	TOTAL SUGAR	FRUCTOSE	GLUCOSE	TOTAL SUGAR	FRUCTOSE	FRUCTOSE
										“GLUCOSE”	TOTAL
Freshly dug tubers	Aug. 30	per cent. 84.6	per cent. 15.3	per cent. 6.7	per cent. 3.6	per cent. 10.3	per cent. 43.6	per cent. 23.4	per cent. 67.1	1.86	0.65
	Nov. 3	79.8	20.1	9.9	5.0	15.0	49.5	25.0	74.6	1.97	0.65
	Dec. 20	81.5	18.4	6.3	5.7	12.1	34.5	31.1	65.6	1.11	0.52
	Jan. 30	81.2	18.7	6.5	6.3	12.8	34.7	33.7	68.5	1.03	0.51
	Mar. 22	83.4	16.5	6.0	5.1	11.1	36.6	30.8	67.4	1.19	0.54
Tubers stored at 32°-35° F. Rela- tive humidity 89- 92	Dec. 20	78.7	21.2	7.9	5.7	13.6	37.4	26.9	64.3	1.39	0.58
	Jan. 30	77.6	22.3	8.0	7.0	15.1	36.0	31.7	67.8	1.14	0.53
	Mar. 22	76.2	23.7	8.5	6.1	14.7	36.0	26.0	62.0	1.39	0.58
Tubers stored at 33°-44° F. Rela- tive humidity 89- 92	Dec. 20	78.0	21.9	8.0	5.9	13.9	36.8	26.9	63.7	1.37	0.58
	Jan. 30	77.9	22.0	8.2	7.3	15.5	37.2	33.1	70.4	1.12	0.53
	Mar. 22	76.7	23.2	8.9	5.7	14.6	38.4	24.4	62.9	1.57	0.61
Tubers stored at 34°-49° F. Rela- tive humidity 74- 86	Dec. 20	76.8	23.1	8.5	5.6	14.1	36.9	24.2	61.1	1.52	0.60
	Jan. 30	74.2	25.7	9.3	7.7	17.0	36.4	29.9	66.4	1.22	0.55
	Mar. 22	69.0	30.9	10.5	7.6	18.2	34.1	24.7	58.9	1.38	0.58
Freshly dug tubers	Aug. 30	83.4	16.5	8.5	3.5	12.0	51.8	21.3	73.2	2.42	0.71
	Nov. 10	78.6	21.3	10.9	5.7	16.6	50.9	26.7	77.6	1.91	0.65
	Dec. 20	80.3	19.6	7.9	5.1	13.0	40.2	26.0	66.3	1.55	0.61
	Jan. 30	82.3	17.7	6.7	5.9	12.7	38.0	33.7	71.8	1.12	0.53
	Mar. 22	82.3	17.6	6.4	5.0	11.4	36.1	28.4	64.6	1.27	0.56

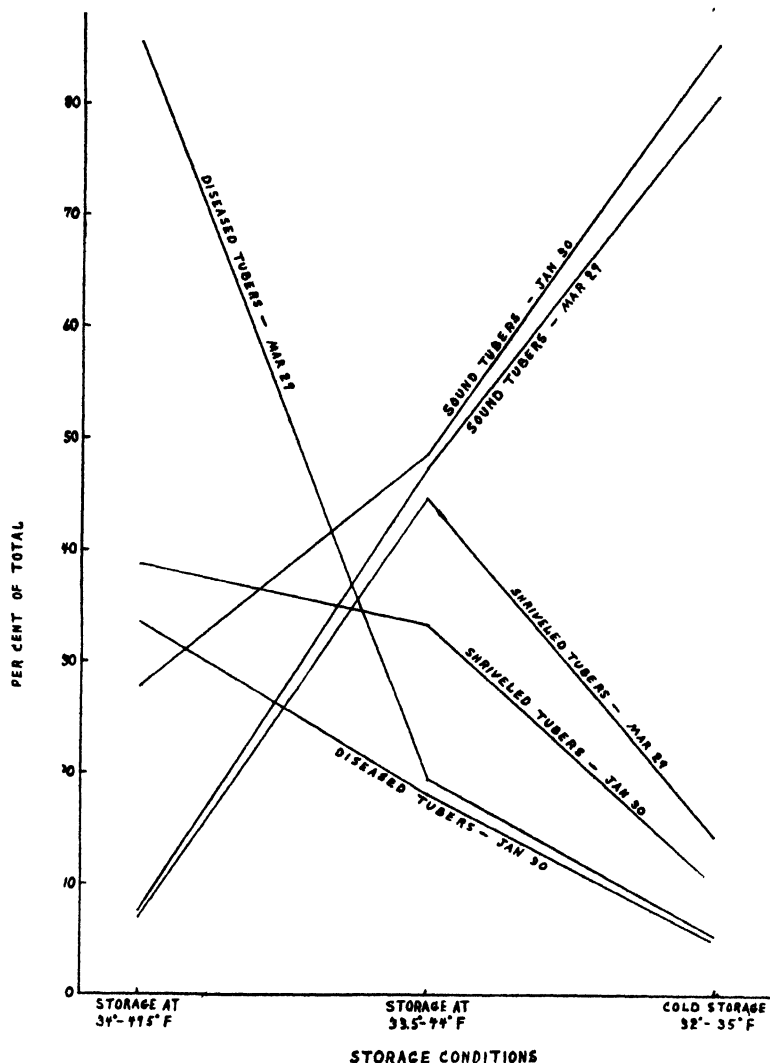


FIG. 1. Per cent. sound, shriveled, and diseased girasole tubers stored under various conditions.

Moisture content.—Prior to the storage period, November 3, there was an increase in dry matter, as shown in figure 2. From then until March 22, the tubers kept in the ground showed a steady increase in moisture. Those kept in a root-cellar at 34°-49° F. and relative humidity of 74-86 per cent. showed a consistent decrease in moisture, totaling 10.8 per cent. Those stored at 33°-44° F. and 89-92 per cent. humidity lost only 3.1 per cent., and those at 32°-35° F. and 89-92 per cent. humidity lost 3.5 per cent.

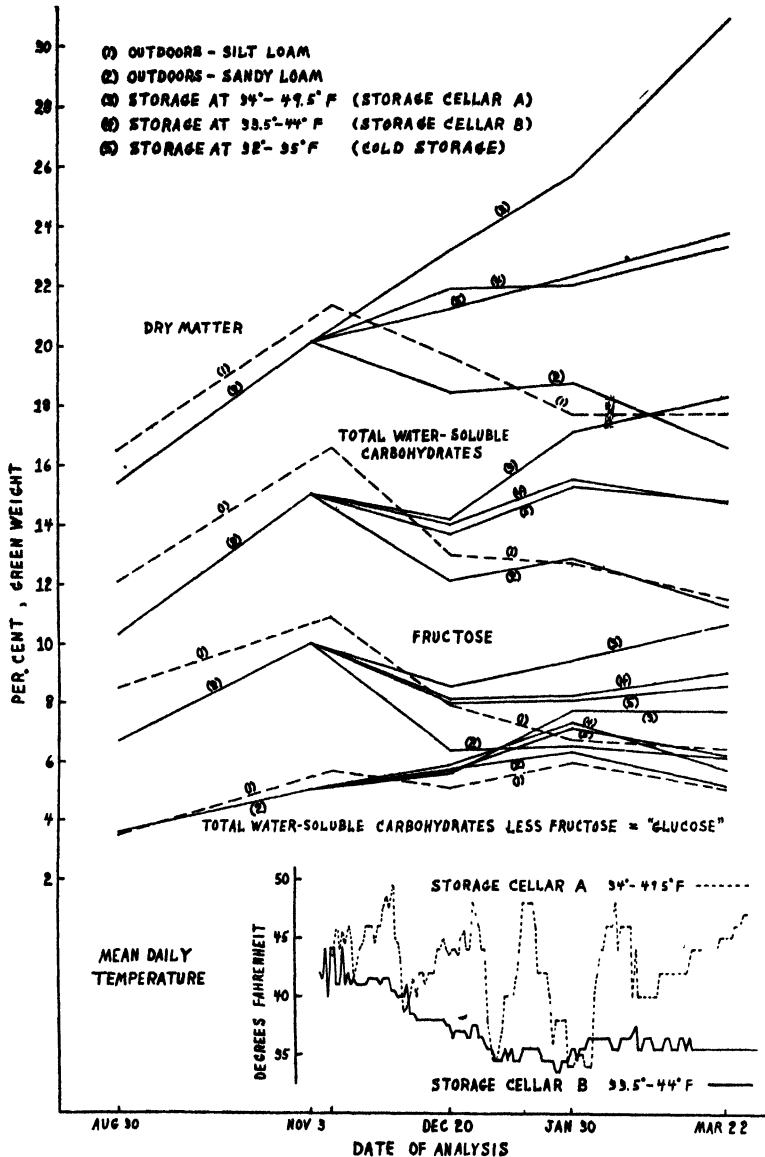


FIG. 2. Variation in dry matter, total water-soluble carbohydrates, fructose and "glucose" in girasole tubers under various storage conditions.
University Farm, St. Paul, Minnesota.

Carbohydrates.—The total water-soluble carbohydrates are built up in the tuber until November, when a maximum is reached, both on the green and dry weight basis. This is shown in table III, and figures 2 and 3.

From November to March there is a total decrease in water-soluble carbohydrate on a dry weight basis. Between November 3 and March 22, there is a loss of more than 10 per cent. of the water-soluble carbohydrates on a dry weight basis. There are minor fluctuations which cannot be explained by the data presented. The rise in water-soluble carbohydrates on January

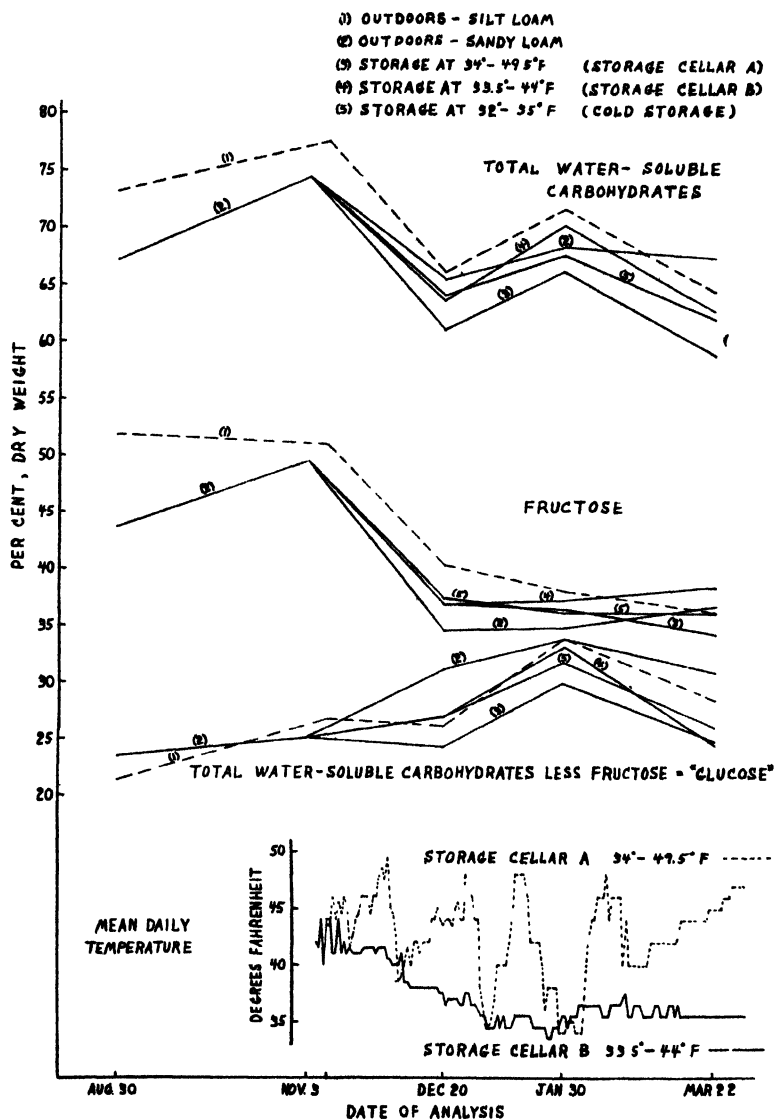


FIG. 3. Variation in total water-soluble carbohydrates, fructose and "glucose" in girasole tubers under various storage conditions.

30 may be due to the seasonal difference in the material. TRAUB (11) has shown that there is a difference in the carbohydrate fractions extractable from apple wood at various seasons of the year. It will be noted that the decline in fructose is consistent, and that the fluctuations in water-soluble carbohydrates is due to other unexplained causes.

In storage the continual loss of moisture apparently has a marked effect upon the percentage of total sugars per unit green weight, as shown in fig. 3. Under the more favorable storage conditions the moisture content and percentage total sugars per unit green weight are more constant.

From the standpoint of possible fructose manufacture the ratios of fructose to other sugars, and fructose to total sugars are of primary importance. During the winter, both in storage and in the field, there is a simultaneous increase in "glucose" and decrease in fructose, although the latter process is the greater as shown in figures 3 and 4. The total sugar content reaches a maximum at the beginning of November, and consequently the best time

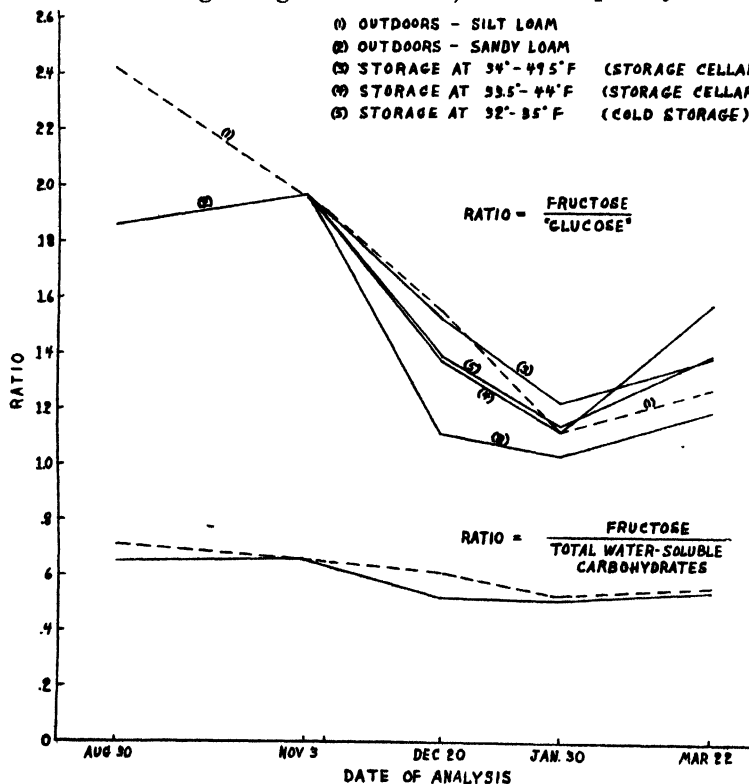


FIG. 4. Ratios of fructose to "glucose" and fructose to total water-soluble carbohydrates, in girasole tubers under various storage conditions.

to utilize the tubers for the maximum fructose yield per unit weight is at this stage when the ratio of fructose to other sugars is the highest.

Conclusions

1. Girasole tubers in storage under the conditions of the experiment have a larger amount of water-soluble carbohydrates per unit green weight than tubers left in the ground over winter.

2. Girasole tubers may be successfully stored as a truck crop, and as propagation stock, at a temperature range of 32°–35° F., and a relative humidity of 89–92 per cent. The optimum conditions for storage, which may or may not approximate the conditions described above, are still to be determined.

3. Tubers stored at temperatures above 40° F. lose moisture rapidly, shrivel, and are subject to storage diseases to such an extent that practically the entire crop so stored is lost by the end of the storage period.

4. From the time of maturity in the fall up to the end of January there is a consistent decrease in the ratio of fructose to glucose, and of fructose to total water-soluble carbohydrates under all conditions studied. From the standpoint of possible fructose manufacture, harvesting and utilization should take place near the time of maturity in November under Minnesota conditions.

5. There is apparently a seasonal variation in the proportion of the water-soluble carbohydrates extractable.

6. Girasole tubers at the time of harvest do not contain appreciable amounts of free reducing sugars.

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 2d ed. Washington. 1925.
2. BIESTER, ALICE, WOOD, M. W., and WAHLIN, C. S. Carbohydrate studies. I. The relative sweetness of pure sugars. *Amer. Jour. Physiol.* **73**: 387–396. 1925.
3. DEERR, NOEL. Relative sweetness of sucrose, levulose and dextrose. *Internatl. Sugar Jour.* **24**: 481. 1922.
4. JACKSON, R. F., SILSBEE, C. G., and PROFFITT, M. J. The preparation of levulose. Dept. of Commerce, Bur. Standards Sci. Papers. no. 519. 1926.
5. NYNS, L. Selective determination of levulose by means of Ost's cupro-carbonate solution. *Chem. Abs.* **19**: 1236. 1925.

6. OLIVER, ROBERTO. The development of methods for the analysis of inulin bearing plants. Masters' Thesis. Univ. of Minnesota. 1927. (In Univ. of Minnesota Library.)
7. PAUL, THEODOR. Begriffsbestimmungen und Masseinheiten in der Süsstoffchemie. *Chem. Ztg.* **45**: 705-706. 1921.
8. SALE, J. W., and SKINNER, W. W. Relative sweetness of invert sugar. *Jour. Ind. Eng. Chem.* **14**: 522-525. 1922.
9. SHOEMAKER, D. N. The Jerusalem artichoke as a crop plant. U. S. Dept. Agr. Tech. Bull. 33. 1927.
10. SPENGLER, O., and TRÄGEL, A. Vergleichende Versuche, betreffend Feststellung des Süßungsgrades von Saccharose und Fructose. *Zeitschr. Ver. deut. Zuckerindus.* 1927: 1-12. 1927.
11. TRAUB, HAMILTON P. Regional and seasonal distribution of moisture, carbohydrates, nitrogen and ash in 2-3 year portions of apple twigs. *Minnesota Agr. Exp. Sta. Tech. Bull.* 53. 1927.
12. WILLAMAN, J. J. The preparation of inulin, with special reference to artichoke tubers as a source. *Jour. Biol. Chem.* **51**: 275-283. 1922.

SEASONAL VARIATION OF THE CHLOROPLAST PIGMENTS IN SEVERAL PLANTS ON THE MALL AT WASHINGTON, D. C.¹

F. M. SCHERTZ

Introduction

The following investigation was undertaken to determine if possible what effect the time of year might have upon the chloroplast pigments. It is of course known that the time of year influences the nutrient elements which are found in the soil and these in turn affect the pigments which are present in the leaves. Plants were chosen, to which no chemical fertilizers were added during the course of the year. It was thought that this experiment might possibly aid in interpreting the results obtained from crop plants which had been fertilized.

Material used

All of the leaves (without petioles) used in these experiments were weighed, ground and extracted immediately after picking. A ten gram sample was used in each case while the extraction and the fractionation of the pigments were done according to methods² already described by the writer. All determinations were made colorimetrically.

The first collection was made from very young leaves as soon as the required amount could be easily obtained and the last collection was made just before frost killed the leaves. The pigments as given in table I are the result of only one determination for each pigment on a single ten gram sample of leaves. Three trees and two shrubs were chosen for the experiment because of the different habits of their growth. The samples were always collected from the same side of the plant, and whenever possible the leaves were picked from the same branch. The data were obtained during the year 1920.

Results

The results of the investigation are presented in table I where the date of the leaf collections and the species of plant investigated are shown.

In looking over the results, it is very difficult to draw any very definite general conclusions regarding the pigments present in these plants. In drawing conclusions from work of this type, one must always consider the

¹ Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

² SCHERTZ, F. M. The extraction and separation of chlorophyll ($\alpha + \beta$), carotin and xanthophyll in fresh leaves preliminary to their quantitative determination. *Plant Physiol.* 3: 211-216. 1928.

TABLE I

THE CHLOROPLAST PIGMENTS (IN MILLIGRAMS PER 10 GRAM SAMPLE OF FRESH LEAVES) PRESENT IN LEAVES FROM SHRUBS AND TREES GROWING ON THE MALL AT WASHINGTON, D. C.

Buzus sempervirens																		
PIGMENTS	YOUNG LEAVES								OLD LEAVES									
	July 1	July 20	August 3	August 17	August 30	September 13	September 27	October 11	October 28*	July 1	July 20	August 3	August 17	August 30	September 13	September 27	October 11	October 28*
	0.93	0.85	0.88	1.00	0.60	1.06	0.75	0.88	0.87	0.96	0.80	0.63	0.83	0.78	0.85	1.03	0.45	0.66
Carotin	1.50	2.00	1.40	2.30	3.00	2.80	2.80	2.80	2.70	1.90	2.40	1.40	1.50	3.00	2.40	2.40	0.50	4.00
Xanthophyll	2.43	2.85	2.28	3.30	3.60	3.80	3.55	3.68	3.57	2.86	3.20	2.03	2.33	3.78	3.25	3.43	0.95	4.66
Total carotinoids	14.1	13.6	18.7	17.0	16.2	18.7	18.0	17.6	21.5	13.6	12.6	15.0	13.1	14.8	13.6	24.1	**	**
Chlorophyll (α + β)																		
Prunus cerasifera																		
Carotin	1.10	1.00	1.10	0.96	0.86	0.73	0.73	0.48	0.32	1.50	0.96	1.00	1.20	0.93	1.00	1.03	0.45	0.66
Xanthophyll	1.60	2.40	2.10	2.10	2.40	2.30	1.70	1.90	2.30	2.80	3.70	3.20	2.80	2.00	2.70	2.40	0.50	4.00
Total carotinoids	3.70	3.40	3.30	3.06	3.26	3.03	2.43	2.38	2.62	4.30	4.66	4.20	4.00	2.93	3.70	3.43	0.95	4.66
Chlorophyll (α + β)	16.0	23.0	18.2	16.0	14.8	14.5	15.6	**	**	21.1	27.5	24.3	23.0	18.7	18.7	24.1	**	**
Chaenomeles lagenaria																		
Carotin	1.50	0.80	1.50	1.40	1.40	1.40	1.40	1.30	1.20	1.40	1.00	1.30	1.10	1.00	0.73	0.96	0.56	1.60
Xanthophyll	3.10	3.50	3.20	3.30	1.00	3.40	3.40	2.60	3.00	3.46	3.10	2.10	2.50	2.10	2.20	2.00	2.40	2.60
Total carotinoids	4.60	4.30	4.70	4.70	2.40	4.80	4.80	3.90	4.20	4.86	4.10	3.40	3.60	3.10	2.93	2.90	2.96	4.20
Chlorophyll (α + β)	25.0	27.5	24.0	20.7	23.5	32.0	32.0	27.5	34.0	17.7	18.2	16.6	16.0	17.6	14.1	18.7	18.7	**

* All of the leaves of these plants were noticeably yellow except those of the *Ginkgo*.

** These chlorophyllin solutions were all of a dirty green color indicating that the chlorophyll had been broken down somewhat.

errors which are ever present in the methods used in making the determinations. The errors which were made in estimating the carotinoids, as has been shown in previous papers which described the colorimetric methods that were used here, are much larger than were the errors made in estimating the total chlorophylls. The chlorophyll data may then be considered of most value.

It is interesting to note that the amount of chlorophyll present in the three trees rises on July 20 and then there apparently is a rapid drop for a month or more and then later a rise in all but the *Ginkgo*. Perhaps the fact that this species belongs to an entirely different group of plants might account for its different behavior. In the case of the boxwood, *Buxus sempervirens*, young leaves (1920 growth) and old leaves (1919 growth) were used in the experiments. The old leaves contained much less chlorophyll than the young leaves. The amount of chlorophyll in both types of leaves apparently increased as the season advanced. The amount of chlorophyll present in the Japan quince evidently increased also as the season advanced. There is no explanation offered as to why the shrubs behave differently than the trees.

It can be said about the carotinoids that two of the trees and the Japan quince contained more pigment at the beginning of the season than did the boxwood and the *Ginkgo*. The maple leaves contained more pigment and there was less variation in the pigment content of this tree during the course of the season than that of any of the other plants investigated. The carotinoids present in *Prunus* decreased to a minimum the first of September and then gradually increased again. The *Ginkgo* leaves showed a gradual decrease in the amount of the carotinoids present in them. On the whole, the old leaves from the boxwood contained less yellow pigments than did the young set of leaves, although the young leaves started the year with less pigment.

The chlorophylls and the carotinoids may be correlated in a few cases. *Acer palmatum* contained the greatest amount of both pigments. The amounts of the green and the yellow pigments present in *Ginkgo* decreased as the season advanced. Both the greens and the yellows decreased and then increased in *Prunus cerasifera*. In a rough way the carotinoids present in the Japan quince parallels the amount of chlorophyll present. In general, it may be said that in every case the amount of carotinoids roughly parallels the amount of chlorophyll present in the leaves.

The investigations of WILLSTÄTTER and STOLL³ have shown that the chlorophyll content of some of the plants which they worked with varied considerably during the growing season.

³ Willstätter, R., and Stoll, A., Untersuchungen über Chlorophyll. 1913.

Table II shows the results of their investigation. Nothing is known regarding the conditions of growth of these plants. These conditions undoubtedly were a very important factor with reference to the amount of chlorophyll which was found in the leaves.

TABLE II

THE AMOUNT OF CHLOROPHYLL PRESENT IN PLANTS AT DIFFERENT TIMES DURING THE GROWING SEASON

PLANT	DATE	MILLIGRAMS OF CHLOROPHYLL PER TEN GRAMS OF FRESH LEAVES
Stinging nettles	June 23, 1911	17.4
	July 20, 1911	22.3
	March 22, 1912	12.6
Plane tree	June 1, 1911	15.3
	July 25, 1911	24.9
Horse-chestnut	June 8, 1911	28.3
	July 22, 1911	34.6

Discussion

In a paper of this kind great care must be exercised to avoid drawing conclusions that are not warranted. There are many chances for error and the investigator must always be on his guard.

To secure data which would be of great value, an investigation such as this one should be continued during the course of several seasons and all meteorological data should be correlated with the quantitative estimation of the pigments. The data from a single season will show only what happened during that one year and of course cannot be relied upon for general conclusions.

Moreover, samples should be analyzed in duplicate so as to detect and eliminate any possible errors in the quantitative separation of the pigments. This would greatly increase the amount of work to be done but it is believed that the results obtained would more than justify the labor required. Caution must be exercised in the taking of samples. The method of sampling must be carefully planned in advance so that it will not be necessary to revise the scheme, for leaves picked from different plants or from different parts of the same plant vary greatly in pigment content. The error in sampling may easily be greater than any errors due to the method of extraction and estimation. Investigations conducted so as to show the pigment content of the leaves from various parts of the same plant would be exceedingly interesting.

In the collection of the leaves, care must be used that the leaves contain unaltered chlorophyll; for if the green pigments are altered in any manner the colorimetric results may not be the true values at all for the chlorophyll. The chief danger with which the investigator must contend is the rapid change during the autumn when the leaves are changing color. Some of the results obtained in this investigation had to be discarded because of autumnal changes which affected the green pigments. Other factors may affect the color of the leaves even during the actively growing season.

Soil conditions also should be carefully noted, for these have been shown to have a very decided influence on pigment formation.

The most valuable data would be obtained if each factor could be artificially controlled. The most important factors influencing the quantity of pigments present are rainfall, soil moisture, nutrient elements in the soil, light, temperature and humidity.

Using Weather Bureau reports, an attempt was made to correlate the effects of temperature, sunshine and rain upon the amount of the chloroplast pigments present in the leaves. It seemed doubtful that any correlations could be established between environmental conditions and the amount of pigments produced from the results of one season's work.

The results obtained in this study show that there is considerable fluctuation in the amount of chloroplast pigments present in green leaves even during the normal growing season. The data presented should lead to a closer study of all the factors which affect the amount of the chloroplast pigments in growing plants.

UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

EFFECTS OF STORAGE ON ALCOHOLIC EXTRACTS OF PLANT TISSUES. AMINO ACID CHANGES¹

JAMES E. WEBSTER

(WITH ONE FIGURE)

Plant chemists, as well as others, are confronted with the necessity of storing much of the material they desire to analyze, since usually a large number of samples are taken at one time. Three major methods are in vogue for this purpose: preserving in alcohol, freezing, and desiccation by heating to various temperatures. Various reasons have been advanced for the use of each, but only in the case of desiccation by heating (3, 4) has extended study been made of the influence of the method upon the distribution of the various constituents. APPLEMAN and ARTHUR (1) report on the effects of storing in alcohol upon certain of the carbohydrates, but apparently little more work has been carried out upon the effects of this very important method upon the extracted constituents. As a result of this scarcity of information it is proposed to begin a series of studies on the effect of storage in alcohol upon the major plant constituents.

While it has been recognized for some time that to make a comprehensive study of the nitrogen fractions in plants, it was necessary to work with fresh material, still many workers have found it convenient to study certain of these fractions in samples preserved in alcohol. Chief among these is the alpha amino acid fraction, and it is this fraction which will be studied in the present work.

Experimental

Preserving.—All of these samples were prepared by boiling the green plant tissue in 95 per cent. aldehyde-free alcohol, to which had been added an excess of CaCO_3 . After boiling for half an hour, the material was cooled and filtered. The alcohol was then expressed from the tissue as thoroughly as possible by hand, and the residue discarded.

Before filtering, a small amount of KNO_3 was added to solution (B) and 1 gram of asparagine was added to the 1800 cc. of solution (D).

Samples.—Solutions (A) and (B) were prepared from commercial samples of spinach and were stored in January. They were alike with the exception of the nitrates added to solution (B). Solutions (C) and (D) were also prepared from market spinach, but these were stored in August. They were alike excepting for the asparagine added to solution (D). Solu-

¹ Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

tion (E) was prepared from young alfalfa, and was started in September.

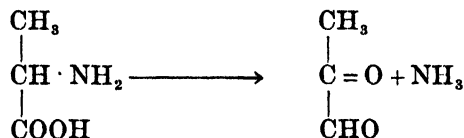
Storage.—Samples (A), (B) and (E) were stored in two liter flasks, sealed to prevent evaporation between samplings. They were placed in a warm spot in the laboratory and exposed to daylight. Samples (C) and (D) were made up to a given volume and then transferred to individual volumetric flasks, sealed and stored in the dark until each determination was to be run.

Analysis.—One hundred-cc. samples of the extracts were evaporated to a small volume several times on a water-bath to remove alcohol, then transferred to 50-cc. volumetric flasks and 2-cc. aliquots were used for each determination. The micro Van Slyke apparatus was used and the procedure outlined in Mathews Physiological Chemistry followed. The results are expressed in terms of mg. of amino nitrogen in 2 cc. of solution and are shown in fig. 1. No allowance was made for ammonia or amide nitrogen in calculating the percentages.

Discussion

In reviewing the graph it is at once apparent that there is a marked decrease in the percentage of amino nitrogen in every case. It is also apparent that the decrease is not uniform in every case; in fact, in sample (C) where the solution was stored in individual flasks there is considerable fluctuation, indicating that the solution was acting differently in the various flasks. It is also worth noting that solutions (B) and (E), high in nitrates, show the greatest changes. However, nitrates were determined on (B) along with the amino acid determinations and showed no change. This seems to indicate, then, at best, only a casual relationship.

Very probably the reaction takes place in the same manner as that occurring in a water solution as shown by DAKIN and DUDLEY (2) in which they were able to demonstrate the formation of ammonia from amino acids as shown in the following equation:



If this represents the true reaction taking place, we would expect an increase in the reducing power of the solution and an increase in ammonia. This problem is to be started at an early date and perhaps then we can say more regarding the mechanism of the change. There is also the possibility of some enzyme action bringing about a deaminization, although this is contrary to the common belief that preservation in alcohol stops enzyme action.

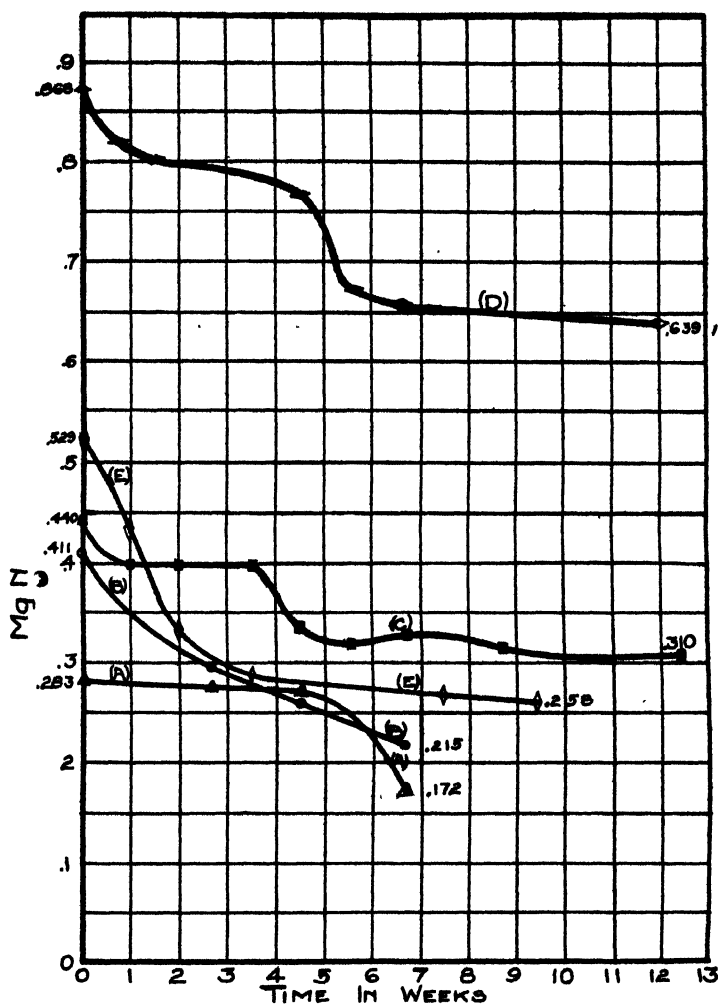


FIG. 1. Graphic representation of the changes in amino acid nitrogen of plant extracts during storage in alcohol.

- (A) Spinach extract, stored in January, in warmth and light.
- (B) Spinach extract + nitrate (KNO_3), stored as (A).
- (C) Spinach extract, stored in August, in darkness.
- (D) Spinach extract + asparagine, stored as (C).
- (E) Young alfalfa extract, stored in September, in warmth and light.

Neither should one overlook the possibility of certain substances catalysing the changes. Work on the influence of nitrates, phosphates, and possibly other groups, will be reported later.

Summary

We are able to conclude from this work that there are some, possibly many, changes taking place when alcoholic solutions are stored for long periods; if this applies as well when the solutions are allowed to remain in contact with the plant material, we must be very careful in drawing conclusions from the analysis of such samples.

The author desires to express his appreciation to Dr. BURRELL, of Ohio State University, for his timely suggestions on this problem.

DEPARTMENT OF AGRICULTURAL CHEMICAL RESEARCH,
OKLAHOMA AGRICULTURAL AND MECHANICAL COLLEGE.

LITERATURE CITED

1. APPLEMAN, C. O., and ARTHUR, J. M. Carbohydrate metabolism in green sweet corn during storage at different temperatures. Jour. Agr. Res. 17: 137-152. 1919.
2. DAKIN, H. D., and DUDLEY, H. W. The interconversion of α -amino acids, α -hydroxy-acids, and α -ketonic aldehydes. Jour. Biol. Chem. 15: 127-142. 1913.
3. LINK, K. P., and SCHULZ, E. R. Effects of the method of desiccation on the nitrogenous constituents of plant tissue. Jour. Amer. Chem. Soc. 46: 2044-2050. 1924.
4. ———, and TOTTINGHAM, W. E. Effects of the method of desiccation on the carbohydrates of plant tissue. Jour. Amer. Chem. Soc. 45: 439-447. 1923.

X-RAY PHOTOGRAPHY OF MINERAL ACCUMULATIONS IN PLANTS

C. L. CRUTCHFIELD

(WITH SIX FIGURES)

The accumulation of mineral salts in plants has heretofore been demonstrated mainly by microchemical means, a method which is not always satisfactory for quantitative ends, since some of the minerals may be masked through organic combinations, and since the amount of color produced, in reactions dependent on colors, is not necessarily proportional to the amount of mineral present in the tissues.

In recent years HOFFER¹ and his coworkers have used the microchemical method to demonstrate the accumulation of iron and other elements in the nodal tissues of *Zea mays* in connection with corn root rot problems. The writer has employed an entirely different method, and has been able to demonstrate such mineral accumulations in the nodal tissues of sugar cane, *Saccharum officinarum*, with results that offer beautiful confirmation of HOFFER's discoveries with the microchemical methods in corn.

The purpose of this paper is to present a few facts concerning the method of detecting mineral accumulations by means of X-rays. It is hoped thereby to stimulate more work along this line, and to encourage the use of this method in studies involving mineral deposition, and mineral translocation in plants. As time goes on X-rays have found an ever widening field of usefulness in science, industry, and medicine. It seems quite probable that many other uses for these rays in biological work may be found.

GEORGE L. CLARK, in his "Applied X-Rays," mentions several of the less well-known applications, and says: "Although X-rays because of their short wave-length are much more able to penetrate matter than ordinary light, still they are differently absorbed by different substances; that is to say, all materials are not equally transparent to X-rays." This fact is the basis of the science of radiography. Broadly defined, the technique consists in passing a beam of X-rays through the object to be examined, and by means of a fluoroscope, or preferably a photographic plate, record the varying intensities of the emergent rays, thereby obtaining a shadow picture of the interior of the object. The first practical uses of the X-rays were probably of a radiographic nature, and today radiography is a most useful tool for the medical and industrial diagnostician.

¹ HOFFER, G. N., and CARR, R. H. Accumulation of aluminum and iron compounds in corn plants and its probable relation to rootrots. Jour. Agr. Res. 23: 801-824. 1923. See also, HOFFER, G. N. Testing corn stalks chemically to aid in determining their plant food needs. Purdue Agr. Exp. Sta. Bull. 298. 1926.

The writer has simply employed the essential quality of the X-rays of being differentially absorbed by different substances, in detecting the localization of mineral depositions in plants. In developing the technique, some substances other than those found abundantly in plants were used, mainly to afford substances differing in opacity to the X-rays. Some of the chemicals used are quite opaque to X-rays, others relatively easily penetrated.

In performing the experiments, solutions of 1-2 per cent. strength were used, of the following salts: Ferrous sulphate, aluminum chloride, potassium sulphate, sodium chloride, lead nitrate, and barium chloride. The heavier metals, lead and barium, are quite opaque; following these are iron and aluminum, relatively considerably less opaque; finally, sodium and potassium are very easily penetrated by X-rays.

Normal average sugar-cane sticks were cut at the surface of the soil, the cut end placed at once into a beaker containing one of the mentioned solutions; control sticks were similarly placed in tap water. All sticks were allowed to remain in the solutions for about 48 hours before they were photographed. During this time, continuing transpiration and existing saturation deficits caused the absorption of some of the solution, which could easily be detected from the decrease of volume of solution remaining in the beakers.

The sticks were now laid across the film-holder containing an X-ray film, and were exposed to X-rays emanating from a Coolidge X-ray tube. In order to secure good photographs, the rays must be adjusted to a suitable penetrating power, and the time of exposure must also be adjusted to the results desired. The photographs reproduced in this paper, were made with a voltage of only about 27500, with 10 milliamperes of current. The exact voltage used depended somewhat upon the thickness of the sticks of cane. The time of exposure was usually about 3.5 minutes. The cane was placed as close to the film-holder as possible in order to obtain clear sharp images. In any given case the investigator will find it necessary to carry on preliminary experiments with different milliamperages and voltages with each object of research, to obtain the best results. In general, the voltage will be much lower than is easily obtained in most makes of apparatus, the average medicinal unit being designed for higher voltages.

The distance from the X-ray tube to the film is, of course, very important since the length of exposure varies with the distance. The photographs shown here are the results of a preliminary study. In fig. 1 is shown a stick which was allowed to take up barium chloride through the cut end. The barium was deposited most heavily at the node, or under the buds. Not only are the nodal deposits shown, but also the vascular system is clearly outlined. It shows better in the photograph than in the reproductions from it. One can see some of the root bands also, rather easily.



FIG. 1. Sugar cane stick, photographed by X-rays after having taken up barium chloride. Dark bands show nodal accumulation of BaCl_2 .

Fig. 2 was made from the photograph of a stalk allowed to absorb lead nitrate solution. The control stick is at the left. The root bands show very



FIG. 2. X-ray photograph of sugar cane sticks; at the right, treated with lead nitrate; at the left, the control. The lead nitrate is very prominent in the root bands.



FIG. 3. Nodal accumulation of iron, from FeSO_4 . Improperly exposed.

clearly here, and the vascular system in the internodal region is brought out quite well. In the control sticks two buds are seen at the nodes. Both figures 1 and 2 are made from stalks containing the most opaque salts used. Lead and barium are not very prominent constituents among the ash elements of plants, though probably never completely absent. Barium is rather universally distributed in soils and plants, but these elements do not occur in such abundance as to show up in the nodes of the control sticks. Iron and aluminum, however, are nearly always fairly abundant in soils. In fig. 3 is shown a photograph from an improperly conducted exposure of a stalk which had been treated with FeSO_4 . The nodal accumulation, in spite of the improper exposure, is clearly shown, and even the vascular bundles can be seen. Better examples of iron accumulation are shown in fig. 6.

The lighter elements, potassium and sodium, are more readily penetrated by X-rays, and so appear to clear up the nodes. In fig. 4 we see a stick which was allowed to take up NaCl . The control stick, to the left, contrasts strongly with the treated one. The latter seems to have deposited the sodium salt sharply at the node. One might question whether it is the sodium, or chlorine, or both that are involved in the deposition. In the treated stick (right) one can see some spots of lighter color. These were found, on cutting the sticks, to be the cavities produced by a stalk borer.

In fig. 5 the control stick is to the right, and the treated stick (left) was allowed to absorb K_2SO_4 . This also seems to clear out the nodal tissue, and to make it less dense than normal. The proper interpretation of this clearing of nodes by Na and K salts probably is that the sodium or potassium displaces some heavier metals, as Fe and Al, that normally are deposited in that region, and so prevents their union with the organic constituents of the nodes. These X-ray results are again in harmony with HOFFER's results in which he claims that potassium prevents the over-accumulation of iron or aluminum in the nodes of corn.

The final figure 6 is most interesting. These stalks were not allowed to absorb solutions, but were taken from soils in which the soluble mineral content was high. The photographs show what can be accomplished in checking up on soil experiments. The small stick to the right is a young cane grown on a soil containing high available aluminum, while the two to the left of it are from a soil with high available iron. The stalks from the iron containing soil showed slight indications of iron injury, but yet growth was practically normal. Tests made for iron and aluminum by other methods did not show them present, but the X-ray photographs clearly show the nodal accumulations. This method may therefore exceed in sensitivity the microchemical methods of detecting these accumulations. A very interesting feature of the stalks grown on the iron-rich soil is the clear indication in

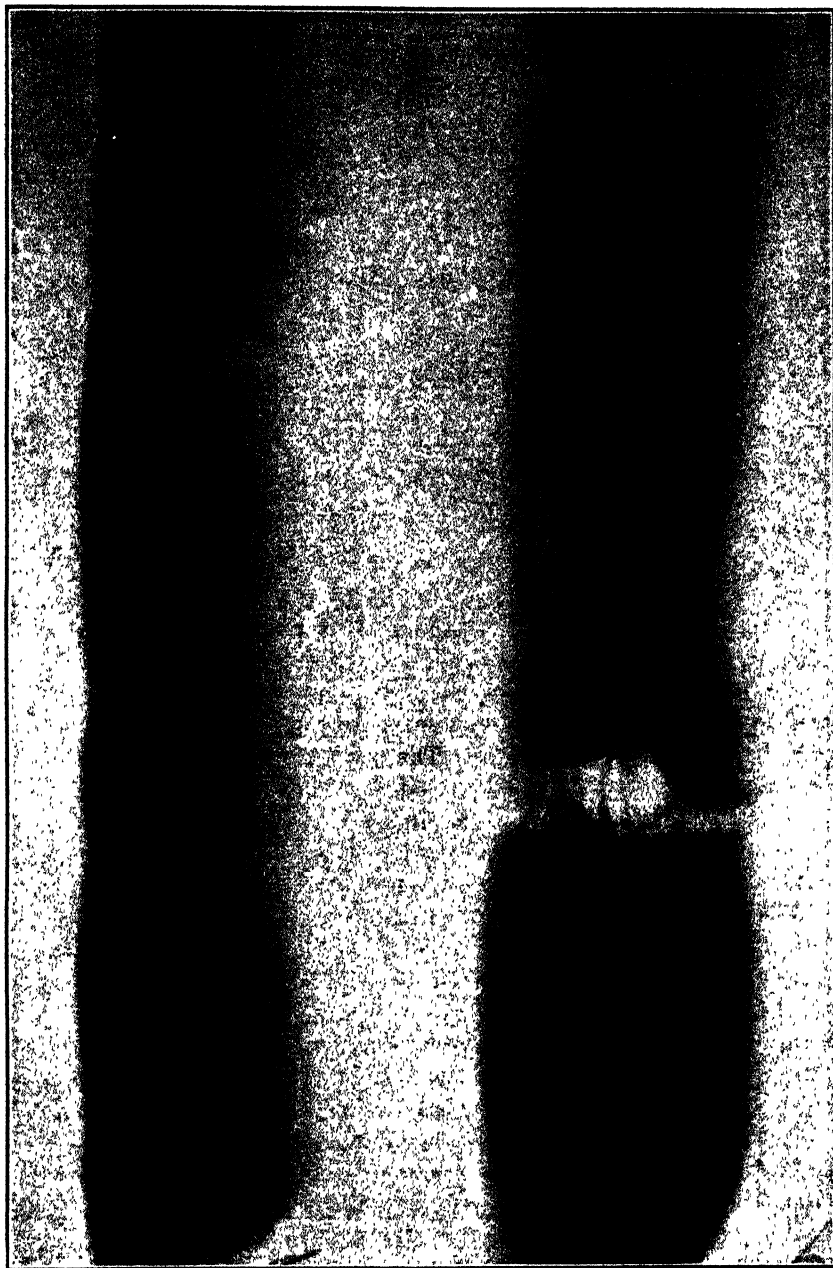


FIG. 4. Sugar cane sticks: Right, after exposure to NaCl, nodal accumulation being indicated by clearer nodal tissue; left, control. Spots in the experimental stick are due to a borer.



FIG. 5. Sugar cane sticks: Left, treated with FeSO_4 ; right, control. Nodal accumulation is indicated by clearer nodal tissue.

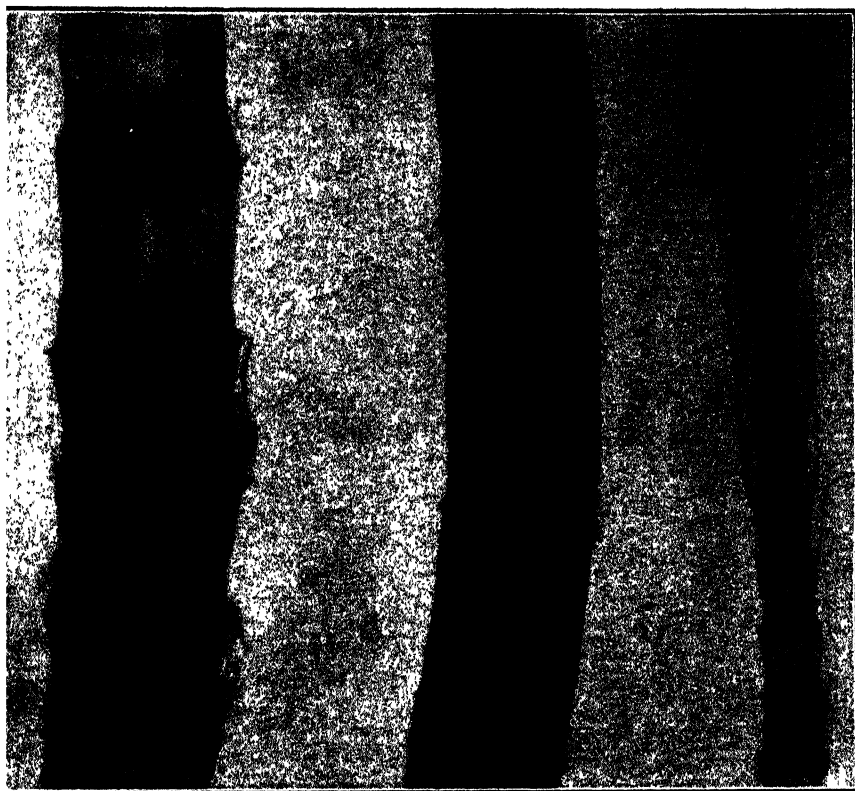


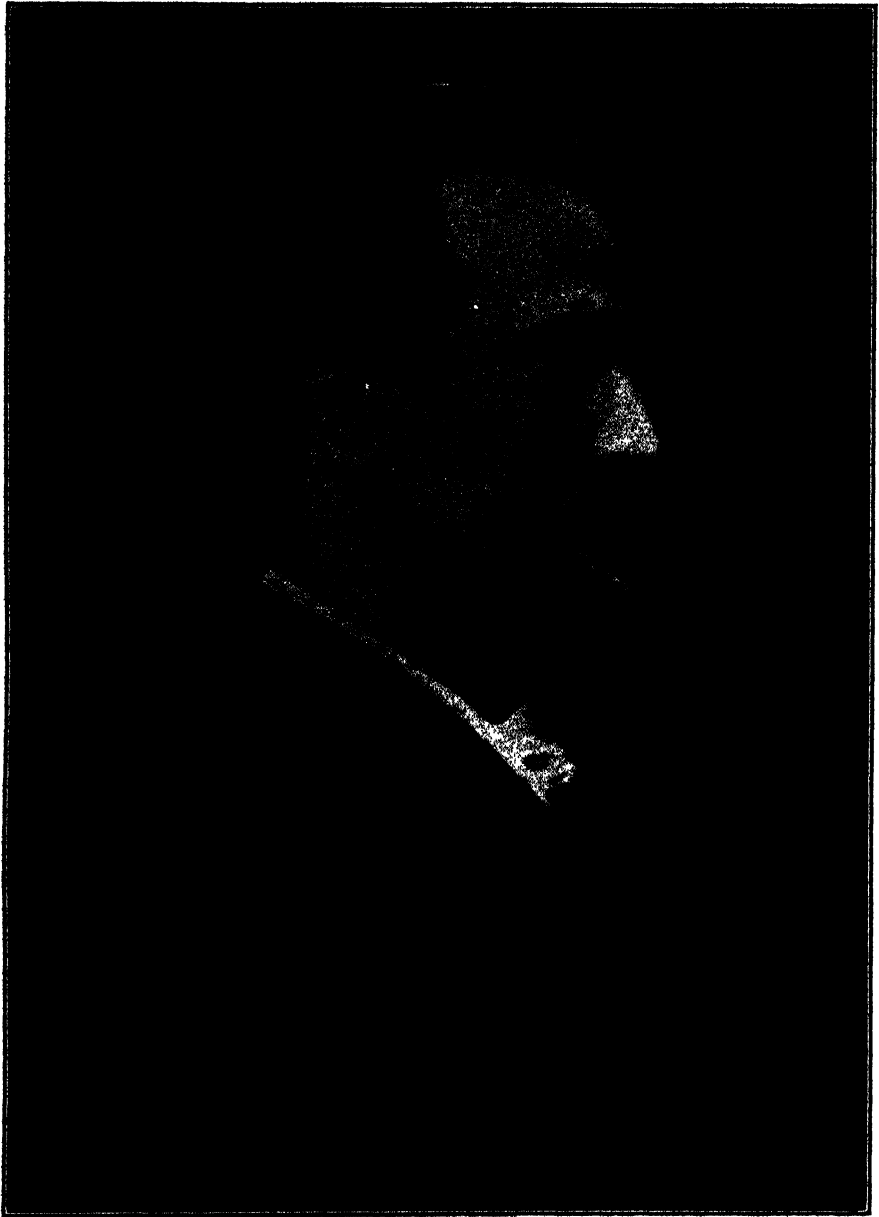
FIG. 6. Sugar cane sticks grown on soils with high available minerals. Right, grown on high available aluminum soil. Center and left, on high available iron soils. The nodal accumulations are easily seen, as well as the vascular tissue of internodes. See text for additional details.

the middle stalk of fig. 6 of three bands of iron deposition at the nodes, one across the central part of the node, and one on either side. Professor ILOFFER, who has examined these photographs, has found similar banded nodal deposits by microchemical means.

Additional work is in progress with the X-ray method in the hope that some of the problems connected with the translocation of salts in plants may be solved. It ought to be possible to determine whether xylem or phloem is mainly concerned in translocation of salts by a study of localization of minerals in plants which have absorbed the salts through unbroken root systems. Space does not permit the inclusion of photographs of some other plants, such as corn, tomato, and some quite good ones of *Selaginella* which seem to show the structure of the vascular system very well.

In conclusion, the writer desires to express his thanks and appreciation to Mr. W. S. KENDRICK, of the Victor X-Ray Corporation, Chicago, for the loan of apparatus, and the use at times of their X-ray equipment and dark room, in connection with the more recent work. He is also indebted to Professor CHARLES A. SHULL for assistance in preparation of the manuscript.

HONOLULU, HAWAIIAN ISLANDS.



JULIUS VON SACHS

BRIEF PAPERS

JULIUS VON SACHS

(WITH ONE PLATE AND ONE FIGURE)

JULIUS VON SACHS has been called the Father of Plant Physiology both on account of his importance in the development of the science and because he was the first teacher to hold a position which recognized plant physiology as a separate subject in university teaching. An estimation of the important position of SACHS in the development of plant physiology was given in a review of his life by F. NOLL in the *Naturwissenschaftliche Rundschau* 12: 460-464, 472-475. 1897. This was in part translated by J. M. COULTER in the *Botanical Gazette* 25: 1-12. 1897. GOEBEL also gives a review of his life in a supplementary volume of *Flora* for 1897. This was translated in *Science Progress* 7: 150-173. 1898.

The life time of SACHS covers a period of rapid growth of plant physiology. In his laboratories there developed many students who spread the zeal of their teacher and who led to the development of their new science. Among these may be mentioned PFEFFER, KLEBS, DEVRIES, BARANETZKY, BREFELD, MILLARDET, MOLL, NOLL, PRANTL, STAHL, ELFFVING, FRANCIS DARWIN, GODLEWSKI, VINES, MARSHALL WARD, GOEBEL, G. KRAUS, MÜLLER-THURGAU, and many other prominent names. SACHS was considered the grand old man of plant physiology by the many German and foreign students of PFEFFER's laboratories. An introduction to him was a high honor. The stimulation of research in plant physiology in the United States owes much to the introduction of his textbooks.

JULIUS was the third son of GRAVEUR SACHS, born at Breslau on October 2, 1832. His father was an engraver by trade, and from him young SACHS learned perfectly the art of delineation, accuracy of line and color, which was of the greatest value to him in gaining a livelihood during the lean years of his student life and also later when he became a teacher. As a child, SACHS had for companions the sons of the physiologist, PURKINJE, and no doubt he was much influenced by this association. Early in boyhood he made collections of plants and was expert in their classification. On many field excursions with his father he was encouraged in botanical collections in which he took great pride. He gave much of his time between the ages of thirteen and sixteen years to drawing and painting the flowers, fungi, and other specimens which he collected. When he was sixteen years old, his father died, and the next year both his mother and a brother died of the cholera. From this time on, SACHS had to earn his

own education by means of the trade which he had learned from his father.

In the Gymnasium from 1845 to 1850 SACHS enjoyed especially the natural sciences. He made a collection of skulls, and wrote a monograph on the crayfish. His natural science teacher, KROBER, solemnly warned him against devoting himself to the natural sciences.

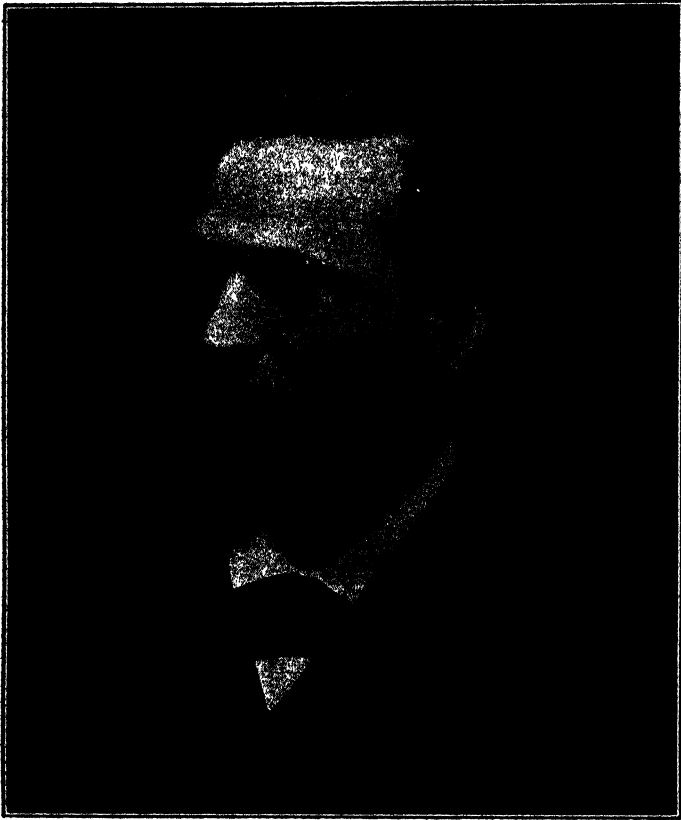


FIG. 1. JULIUS VON SACHS.

Upon the death of both parents, SACHS was taken into the family of PURKINJE who had accepted a professorship at the university at Prag. He completed the courses of the Gymnasium and was admitted to the university in 1851. In Prag he published a Bohemian scientific journal, *Ziva*, and to this journal he made a number of scientific contributions.

Upon the completion of his university course in 1856, SACHS began to devote his time to plant physiology, working especially upon seed germination, the digestion and transport of nutrients, and the development of plant

organs. In April, 1859, upon the recommendation of Hofmeister, Sachs became assistant to Stöckhardt at Tharand. He was quickly advanced to take charge of the laboratory of experimental plant physiology. In 1861 he was elected head of the experiment station for plant physiology at Chemnitz, but went instead to a professorship in the Poppelsdorf Agricultural Academy of the University of Bonn. The six years at this academy were most fruitful for research, and here also was published his Handbook of Experimental Plant Physiology. In 1867 Sachs was called to Freiburg to the chair vacated by the resignation of DeBary, but one year later he succeeded Schenk at the University of Würzburg. Here he had at his disposal a large building for his botanical institute. His laboratory became the center of plant physiological research although the number admitted at one time was limited to ten students. Sachs published in 1868 his Lehrbuch, in 1875 a History of Botany, and in 1882 his Lectures upon Plant Physiology, all books outstanding even to the present day. He died at Würzburg on May 29, 1897.—R. B. HARVEY, *University of Minnesota*.

AN INVADING POTATO SPROUT

When potatoes are grown in fields infested with Couch Grass (*Agropyron repens*), it is not uncommon to find some of the tubers transfixd by the hard, sharply pointed rhizomes of this persistent grass. A growth phenomenon similar to this in appearance, yet very different in nature, was recently observed by the writer; and as this phenomenon is apparently a very unusual one, it deserves to be included in our botanical records.

A sack of potatoes had been stored in a cellar during the winter months, and toward the close of this period as the days grew warmer and the general level of temperature began to rise, the buds of the tubers became active, and vigorous sprouts were formed. On one of these tubers a sprout was observed that had behaved in a most unusual manner. In its growth it encountered a neighboring tuber, in the side of which a small cavity about 5 or 6 mm. in depth had been made by a wire worm. The growing point of the sprout entered this cavity and then continued its growth into the storage tissue of the potato, until it finally emerged on the opposite side. When it was first discovered the sprout had attained a length of 6.5 centimeters, and a diameter of 8 mm.

The accompanying photograph, figure 1, shows the position of the sprout imbedded in the tuber just as it was found, except that a portion of the tissue has been removed so that the full length of the sprout may be seen. At no point was there any evidence of a coalescence of the cells of the sprout with those of the tuber through which it grew, but the turgid sprout fitted very closely into the tunnel it had made. The surface of the tuber tissue

surrounding the sprout was very smooth, and at the point of emergence there was not the slightest indication of a rupture of the tissues, as would probably have been the case had the expanding cells of the sprout broken through by the mechanical force of growth.

The general appearance of the tissue of the tuber suggested that the cells had been digested by the elongating sprout which was able in this way to excavate the tunnel through which it grew. This further suggests the possibility of a sprout arising on one potato being able to procure a part or all of its nutrition from an adjacent tuber—at least under such circumstances as those here recorded.—P. D. STRAUSBAUGH, *West Virginia University*.

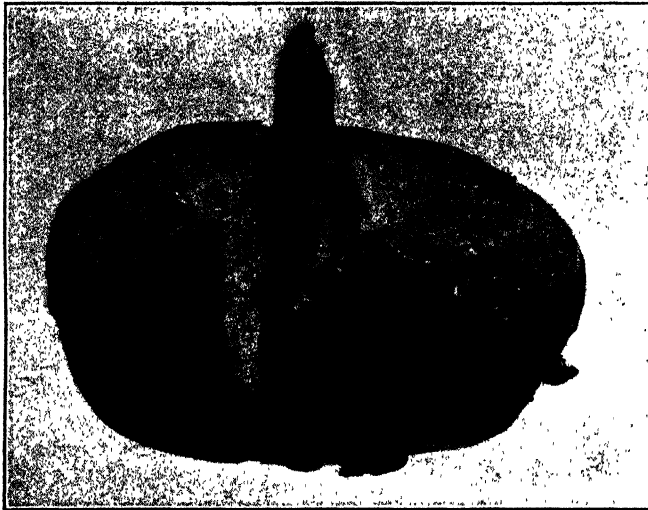


FIG. 1. Potato tuber, penetrated by sprout from a neighboring tuber. The sprout apparently digested its way through the invaded tuber.

SCHLOESING'S EXPERIMENTS ON THE RELATION OF TRANSPIRATION TO THE TRANSLOCATION OF MINERALS

In the last fifteen years a number of papers have been published showing at least partial independence of transpiration and mineral absorption. HASSELBRING (2) found that tobacco grown under shade had as high ash content as plants grown in the open. MUENSCHER (3) found that doubling the transpiration of barley by decreasing the humidity had no effect on ash absorption. Doubling the transpiration by differences in light intensity nearly trebled the absorption of ash but the ratio of ash to dry matter was

not affected. CURTIS (1) has attempted to show that solutes are translocated primarily in the phloem where their movement would of course be unaffected by transpiration.

In spite of this accumulation of evidence, however, SCHLOESING's experiment (4) published in 1869 has been so generally accepted in the older texts that its influence persists very generally among teachers of plant science. This note points out certain details in the experimental procedure used by SCHLOESING which have been overlooked in translation, and which would seem to invalidate the conclusions commonly drawn from the data, thus removing the conflict between this and more recent experimental work.

SCHLOESING grew four tobacco plants in large pots, one under a bell jar shaded with muslin for four weeks, and three in the open air for six weeks. The plant under the bell jar transpired 7.9 liters, or 1976 ml. a week while the average transpiration of the exposed plants was 23.3 liters, or 3883 ml. a week.

The dried leaves of the protected plant contained 13.0 per cent. of ash and of the exposed plants 21.8 per cent. at the end of the experiment. After correcting for dry weight and ash content at the beginning of the experiment the total gain of ash was 4.50 and 6.41 grams respectively. The data are claimed to prove the dependence of mineral movement on transpiration.

An examination of the conditions of the experiment shows that the protected plant was not only shaded but received additional CO_2 in undetermined quantities. The results of the experiment can without difficulty be attributed to this difference in the CO_2 supply of the two lots of plants. For example, the rate of gain in dry weight was 10.0 grams a week for the plant receiving additional CO_2 and only 4.9 grams a week for the checks. There was also a rapid accumulation of starch in the plant under the bell jar to a total of 19.0 per cent., while the leaves of the exposed plants contained only 1.0 per cent. of hydrolyzable polysaccharides. The lower per cent. of ash is in large part due to the very rapid accumulation of carbohydrates in the protected plant.

If we consider the rate of ash assimilation we find that the covered plant gained 0.57 grams of ash for each liter of water transpired while the exposed plants gained only 0.28 grams for each liter of transpiration. Also the gain per week in the covered plants was higher in total ash and in all determined elements except chlorine, silica, and iron, and was very much higher in phosphorus and nitrogen.

Summary

The more or less classic experiment of SCHLOESING has been given an unjustified importance in establishing the dependence of mineral movement in the plant upon transpiration. The data are better interpreted

as showing the effects of increased CO_2 rather than of decreased transpiration.—W. E. LOOMIS, *Iowa State College*.

LITERATURE CITED

1. CURTIS, O. F. Studies on the tissues concerned in the transfer of solutes in plants. *Ann. Bot.* **39**: 573–585. 1925.
2. HASSELBRING, HEINRICH. The relation between the transpiration stream and the absorption of salts. *Bot. Gaz.* **57**: 72–73. 1914.
3. MUENSCHER, W. C. The effect of transpiration on the absorption of salts by plants. *Amer. Jour. Bot.* **9**: 311–329. 1922.
4. SCHLOESING, TH. Végétation comparée du tabac sans cloche et à l'air libre. *Ann Sci. Nat. Bot. Ser. V* **10**: 366–369. 1869.

NOTES

Fifth Annual Meeting.—The fifth annual meeting of the American Society of Plant Physiologists, held at New York on December 28, 29, and 31, 1929, marks an important milestone in the development of the society. Although the attendance was reduced by the widespread influenza epidemic, and the meeting handicapped by the unexpected absence of both president and vice-president, temporary officers were chosen, and the sessions held as scheduled. The programs were interesting and valuable, as usual, and every one must have felt repaid for being present. The most encouraging feature of the meeting was the detailed report of the secretary-treasurer, Dr. H. R. KRAYBILL, who analyzed the gains in membership and financial support during the last year. The society continues to gain in numbers at a steady rate, and financial support has increased more rapidly than at any time since organization. The report of the condition of the finances makes it evident that PLANT PHYSIOLOGY has not incurred a deficit since its first year; moreover, the unsold volumes now have a value considerably larger than the subsidy which was provided for the first year of publication. It is remarkable that a journal of the high standards of PLANT PHYSIOLOGY could become self supporting within the first year of its existence, but the secretary's report shows that this is the fact. Good fellowship marked all of the meetings, and was enjoyed by all. The facilities provided by Columbia University for the meetings were much appreciated.

Life Membership Award.—The committee appointed to choose the third CHARLES REID BARNES life member, of which Dr. W. M. ATWOOD was chairman, selected Dr. HERMAN AUGUSTUS SPOEHR, of the Coastal Laboratory of the Carnegie Institution, Carmel, California, for this honor. The selection is a very happy one. Dr. SPOEHR has made very valuable contributions to our knowledge of plant physiology. Unlike many biochemists, he knows the living plants thoroughly. His work on carbohydrate metabolism, on respiration, and on photosynthesis, is outstanding work. Dr. SPOEHR has been connected with the Carnegie Institution since 1910, and became assistant director of the Coastal Laboratory in 1926. His excellent summary of the process of photosynthesis published in 1926, is the best account in the English language. All members of the Society will feel that the Society has been honored by this award to Dr. SPOEHR. He becomes the third link in the chain of life members who form a living memorial in honor of Dr. CHARLES REID BARNES, first professor of plant physiology at the University of Chicago.

Stephen Hales Award.—It was expected that the first STEPHEN HALES Prize would be awarded at the New York meeting. However, the committee having this matter in charge, found that it would be advisable to postpone its decision for a time, and announce the first award at the Des Moines meeting in 1929. While this decision may have been a disappointment to some, the delay has some valuable aspects. Certain matters of policy have not been clearly crystallized, and the development of a satisfactory certificate has presented some complications. The additional time will make it possible to meet every problem connected with the award in a satisfactory manner.

Forest Tree Seeds.—Notes have appeared in past numbers of PLANT PHYSIOLOGY regarding sources of seeds for investigational purposes. In this connection, Conyers B. Fleu, Jr., of Germantown, Philadelphia, Pa., has been mentioned, and Otto Katzenstein and Co., of Atlanta, Ga. There are also other sources of seeds of guaranteed origin and quality. The Associated Foresters, Ltd., of Calgary, Canada, advertise seeds of known origin, and will supply along with the seeds, data as to site, stand, and climatic conditions under which they grew. The Brown Co., Berlin, New Hampshire, also advertise forest tree seed of certified origin. It is believed that any of these companies will exercise painstaking care in furnishing seeds for research on the physiology of germination of forest tree seeds.

The U. S. Forest Service has a mimeographed list of forest tree seed dealers, together with a list of the most important U. S. tree species, giving the average number of seeds to the pound, and the dealers who are in position to supply seed of each species.

The Rev. J. Farnsworth Anderson, Glenn Hall, Leicester, England, advertises fresh seed of perennials from all over the world. It is hoped that those needing seeds will find these addresses valuable.

Research Laboratory at Minnesota.—A low temperature research laboratory has been completed at the Minnesota Agricultural Experiment Station for the Section of Plant Physiology. The new installation consists of four refrigerated rooms 9 x 10 x 11 feet, a control laboratory, engine room with 15 horse power compressor, and two controlled greenhouses, with potting sheds. Additional greenhouse space is available also, for cooperation with the Division of Agronomy and Plant Breeding on the winter resistance of field crops, and with the Section of Plant Pathology on pathological physiology.

The refrigeration is by automatic machines of the Lipman type. The rooms are insulated with 12–14 inches of sheet cork. Temperature fluctuations in these chambers is $\pm 2^{\circ}$ C. with the Absolute Contactor Corpora-

tion regulator, $\pm 0.5^{\circ}$ C. when the Harvey type thermoregulator is used. Temperatures and humidities are recorded by resistance thermometers and a recorder of the Leeds & Northrup Co. A recording potentiometer for use with photoelectric cells is available for light records.

Fermentation.—SCHOEN, of the Institut Pasteur, has published a monograph on alcoholic fermentation which has just been issued in English translation. It is an unusually lucid and readable account, and summarizes admirably the present status of our knowledge. Especially commendable is the chapter on the role of H^+ in oxidation processes, which gives the clearest discussion of the nature of oxidation in living cells, which the reviewer has seen anywhere. It is published by Chapman and Hall, of London, translated by HIND, and entitled *The Problem of Fermentation*. The price is 21 shillings.

Colloid Chemistry.—The second volume of *Colloid Chemistry*, theoretical and applied, collected and edited by JEROME ALEXANDER, has appeared from the press of the Chemical Catalog Co., New York. This volume, devoted to Biology and Medicine, contains 57 papers by men of recognized authority in their respective fields, and is a splendid contribution to a fuller understanding of the significance of the colloidal state in living organisms. A few titles will suffice to indicate the unusual nature of this book. There is a discussion of Colloids and X-rays, by Sir WILLIAM BRAGG; Proteins as Colloids, by WOLFGANG PAULI; Inorganic Ferments, by G. BREDIG; The Adsorption of Enzymes, by RICHARD WILLSTÄTTER; Bacteriophage, a Living Colloidal Micelle, by F. D'HERELLE; Colloidal State and Physiological Function, by RUDOLPH HÖBER; The Arrangement and Action of the Colloids of the Plant Cell, by D. T. MACDOUGAL; The Physieal Basis of Life, by EDMUND B. WILSON. An appendix contains the Pasteur Lecture delivered in Chicago by JACQUES LOEB on November 24, 1922, in lieu of a paper which LOEB was to have contributed to the volume.

The book is one that every physiologist must read if he is to keep abreast of modern thought on the processes of living. It is a large volume, over 1,000 pages, and costs \$15.50; but one could easily spend more and get less in value, as books are now selling. All libraries of biological institutions will find it a necessity.

Numerical Data of Biology.—Attention is called to the publication of several chapters from vol. VI, 1923–1924, of the *Tables Annuelles de Constantes et Données Numérique* under the title *Données Numérique de Biologie et de Physiologie et Chimie Végétales*, by E. F. TERROINE and H. COLIN. The tables include bulk chemical compositions of plants; chem-

ical composition of special organs and tissues; content of inorganic, organic and organometallic substances in plants; physical-chemical constants of plant juices; data on gaseous exchanges; and enzyme reactions. It comes in quarto size, 88 pages, from Gauthier-Villars et Cie., 55 Quai des Grands-Augustins, Paris (VI). Bound, the price is 60 francs, 44 francs in brochure form.

Colloid Chemistry of Starch.—The second volume of the *Handbuch der Kolloidwissenschaft*, edited by W. OSTWALD, is a monograph on starch, *Kolloidchemie der Stärke*, by M. SAMEC. It contains 16 chapters, dealing with the general characteristics of starch, molecular structure, construction of starch grains, physical characteristics of starch grains, adsorbent properties, paste formation, solution, properties of solutions of starch, protective colloidal action, adsorption of starch by other substances, changes in starch solutions, precipitation of starch, alkali starch, formaldehyde starch, starch esters, and dextrine. The book brings together in excellent fashion the whole field of starch behavior, laying more emphasis upon the experimental results than upon changing theories of starch constitution. It is published by Theodor Steinkopff, Dresden and Leipzig. In brochure form it costs RM 30, and RM 32, cloth bound.

The Alkaloids.—The first part of the second edition of WINTERSTEIN-TRIER's *Die Alkaloide*, was published by Gebrüder Borntraeger, Berlin, in 1927. This first part contains 356 pages, and presents the alkaloids whose constitution is fairly well known. The aliphatic nitrogen bases, aromatics, acid amides, urea derivatives, and the heterocyclic bases of the pyrrol-pyridine group occupy this volume. It is replete with interest to those investigating the nitrogenous metabolism of plants. In general the natural plant bases receive too little attention from physiologists. The price of this volume is RM 18.

Colloid Chemistry of Protoplasm.—The first volume of a new series of monographs, *Protoplasma-Monographien*, is a book by L. V. HEILBRUNN on the Colloid Chemistry of Protoplasm. It is written in English, although published by Gebrüder Borntraeger, Berlin. Several introductory chapters deal with the morphology and chemistry of protoplasm, and methods of study. Then the problems of viscosity, elasticity, temperature and other effects, salt action and electric charges are considered. Other chapters discuss the action of acids and bases, fat solvents, surface precipitation reactions, a specific colloid chemical reaction peculiar to living organisms, and cell division and protoplasmic activity. The book contains 356 pages, and is a fine contribution to the literature of protoplasm. In paper cover, RM 19, bound in cloth, RM 21.

PLANT PHYSIOLOGY

APRIL, 1929

A STUDY OF THE RELATIONS BETWEEN CHLOROPLAST PIGMENTS AND DRY WEIGHTS OF TOPS IN DENT CORN¹

H. B. SPRAGUE AND J. W. SHIVE

The dearth of information regarding the exact relation between the concentration of the chloroplast pigments (chlorophyll α and β , carotin, and xanthophyll) in higher plants, and growth rates, and between the total quantity of these pigments and the total amount of growth made (10) (12), was the stimulus which started the work here reported. Three series of experiments were conducted, to determine as carefully as possible the amounts of chloroplast pigments present in certain leaves at periodic intervals, as well as leaf areas and dry weights of the plant tops. Dent corn (*Zea mays* L.) was selected for the tests, because of its great economic importance and the availability of practically homozygous seed.

Experimental methods

Three series of plants were grown, series 1 and 2 in the greenhouse in the springs of 1925 and 1926, and series 3 in the field during the summer of 1925. Nebraska 10 and Nebraska 12, two lines of corn that had been self fertilized for 17 generations, were used in all experiments. Schmidt's White Cap, a local open-pollinated strain was included in series 1. The F_1 cross between the selfed lines Nebraska 12 and Nebraska 10, was used in series 2, and the F_1 cross of Nebraska 12 and Nebraska 659 in series 3. The use of selfed lines and of first generation crosses avoided much of the random variation among individual plants that would have been present had ordinary varieties been selected. At the same time, it provided for constant genetic differences between the plants of the different strains. All of the strains chosen appeared normal for color.

The seeds planted were carefully selected for uniformity. Those of the selfed lines and the crosses planted in series 1 and 2 weighed between 0.185 and 0.195 grams, and those of Schmidt's White Cap averaged 0.295 grams.

¹ Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

The seeds planted in series 3 were selected for uniformity of size but were not weighed.

SERIES 1 AND 2

The plants in series 1 and 2 were grown in percolators, as described by ALLISON (1), each filled with 3,328 grams of pure washed quartz sand. The seeds were germinated in sphagnum moss and six seedlings were transplanted to each percolator when the plumules were 2 cm. long in series 1, and 3 to 4 cm. long in series 2. Culture solution was supplied by the constant drip method (9). Beginning 30 days after transplanting in series 1, and 12 days after transplanting in series 2, distilled water was added once daily to the surface of the sand in each percolator just before renewing the solution in the constant drip reservoirs. This largely prevented concentration of the nutrient solution in the sand through water loss by absorption and evaporation. Enough water was added to cause free dripping from the bottoms of the percolators.

The plants in series 1 were supplied with SHIVE's nutrient solution R_5C_2 (1.75 atm.) at the rate of 1 liter per percolator per day. For series 2, TOTTINGHAM'S (13) solution $T_1R_1C_5$ as modified by JONES and SHIVE (3) was used at the same rate. Iron was added to the nutrient solution daily in the form of soluble ferric phosphate. The amount used was varied as needed to maintain a normal green color. For series 1, a total of 446 mg. of iron was added to each culture of Nebraska 12 and Schmidt's White Cap, and 334 mg. to Nebraska 10 during the growth period of 52 days. For series 2, a total of 101.7 mg. of iron was added to each culture of Nebraska 12 and the F_1 (Nebraska 12 x Nebraska 659) while 76.3 mg. were added to Nebraska 10 cultures, during the period of 45 days.

SERIES 3

Series 3 was planted in a well prepared seed bed on May 20. The soil was Sassafras silt loam, to which 400 lbs. of a commercial fertilizer having an analysis of 2 per cent. NH_3 , 12 per cent. P_2O_5 and 2 per cent. K_2O was broadcast before planting. The crop was tilled in the ordinary manner except that care was taken to prevent injury to any seedlings and to prevent weed growth.

SAMPLING

The plants in each series were considered up when the first leaves began to unfold. The ages given in subsequent tables are on the basis of dates when plants came up. Plants were harvested periodically using 12 plants of Nebraska 12 and Nebraska 10, and 2-8 plants of Schmidt's White Cap at each harvest in series 1; and 6 plants per strain per harvest in series 2 and 3.

Leaf areas (leaf expanses) were obtained by means of blueprints and a planimeter for the first three harvests of series 1. All other leaf areas were determined by the formula (4):

$$\text{leaf area} = 0.75 (\text{total length} \times \text{width at widest place})$$

Only the green portions of the blades were used in determining areas.

One leaf from each plant was selected for analysis of chloroplast pigments; namely, the uppermost one which had attained its maximum size and a normal color. The dry weight of each leaf used for pigment analysis was calculated by assuming that the ratio of area to weight was the same for this leaf as for the rest of the leaves on the plant. Although the uppermost fully developed leaves may not have been absolutely representative of all the leaves on that plant, they should have been comparable for all strains at each harvest since the same method of selection was used throughout.

DETERMINATION OF CHLOROPLAST PIGMENTS

The fresh green leaves selected for pigment analyses were thoroughly triturated with sand to permit quantitative extraction. The method used in extraction and separation of the various pigments was that of WILLSTÄTTER (14) as modified by SCHERTZ (7). The chlorophylls, α and β , were obtained together as an aqueous solution of the chlorophyllins, which appeared green by transmitted light. In this work, it was found convenient to use petrol ether instead of the diethyl ether recommended by SCHERTZ as a solvent for the carotinoids; the quantities of these pigments, therefore, were determined from petrol ether solutions.

The amount of each pigment was measured colorimetrically by comparing a solution of the pigment with an artificial color standard in a Duboseq colorimeter. The color standard for chlorophyll was prepared by adding 0.3 cc. of a 0.5 per cent. aqueous solution of malachite green and 11.2 cc. of a 0.5 per cent. aqueous solution of naphthol yellow to distilled water, and making up to 5,500 cc. volume. The carotin color standard was made by adding 3.4 cc. of a 0.5 per cent. aqueous solution of naphthol yellow and 0.5 cc. of a 0.5 per cent. aqueous solution of orange G. crystals to 1 liter of distilled water. The xanthophyll color standard was prepared by adding 3.8 cc. of the 0.5 per cent. aqueous solution of naphthol yellow to 1 liter of distilled water.

The 0.5 per cent. aqueous stock solutions of malachite green, naphthol yellow, and orange G. will keep for a long time in tightly stoppered bottles in the dark (table I). The color standards, however, should be freshly prepared from the stocks every one or two weeks to eliminate any danger of fading of colors. Stock solutions of the dyes should be discarded whenever a sediment appears.

TABLE I

THE STABILITY OF THE 0.5% PER CENT. STOCK SOLUTIONS OF DYES USED IN MAKING COLOR STANDARDS FOR CHLOROPLAST PIGMENTS¹

DYES	FRESHLY MADE	6 MONTHS OLD	8 MONTHS OLD	10 MONTHS OLD	20 MONTHS OLD
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Naphthol yellow ² (Martius yellow) Schultz No. 6	100.0	100.0	100.0	98.0	100.0
Orange G. Crystals ³ ..	100.0	64.5 ⁴	66.7 ⁴	100.0	100.0
Malachite Green ² Schultz No. 495.	100.0	100.0	100.0	100.0	100.0

¹ The stock solutions were diluted until readings could be made accurately with the Duboseq colorimeter.

² Purchased from the National Aniline and Chemical Co., Inc., New York.

³ Purchased from Arthur Thomas Co., Philadelphia.

⁴ Heavy sediment found in solutions.

Each color standard was evaluated in terms of milligrams of the pigment with which it was used. Dr. F. M. SCHERTZ, of the U. S. Department of Agriculture kindly provided a sufficient quantity of pure crystalline chlorophyll (α plus β) to evaluate the chlorophyll standard. Five hundred milligrams of chlorophyll were dissolved in acetone, transferred to petrol ether, saponified with methyl alcoholic potash in the usual way, separated from the ether solution, and made up to volume with water. This solution was then compared with the artificial chlorophyll color standard in the colorimeter. It was found that 10.708 milligrams of chlorophyll converted to chlorophyllins and made up to 1 liter matched the tint and depth of color of the artificial standard.

Evaluation of the carotin and xanthophyll standards was accomplished by extracting and purifying these pigments, and making readings with the colorimeter of the pigments in solution against their respective color standards. The concentration of pure pigment in each solution was determined from aliquot parts of the same solution by means of a König-Martens spectrophotometer at the Bureau of Standards at Washington, D. C. This was accomplished through the courtesy of Dr. D. S. GIBSON of that Bureau and of Dr. F. M. SCHERTZ of the U. S. Department of Agriculture. This quantitative method of measuring the carotinoid pigments has been worked out by SCHERTZ (5, 6). It was found that 1.890 milligrams of carotin per liter of solution were required to match the tint and depth of color of the carotin color standard, whereas 1.537 milligrams of xanthophyll per liter

of solution were required to match the tint and depth of color of the xanthophyll standard.

WILLSTÄTTER and STOLL (14, 15) prepared color standards for chlorophyll by making chlorophyllin solutions from known quantities of pure chlorophyll; and for the carotinoids by making up solutions of known concentrations of the pigment in question, or by making up solutions of potassium dichromate corresponding in color to solutions of a known concentration of the pigment to be measured. The color standards described above proved more satisfactory than WILLSTÄTTER's since they were easily prepared; also the colors were more stable, and were identical with those of solutions of the pigments to be measured. Since pure chlorophyll, carotin, and xanthophyll are difficult to prepare and may not be purchased in the open market, the artificial standards are quite useful when once prepared. The colorimetric method of measuring pigments is satisfactory only if consistent readings may be obtained. When stable color standards, which accurately match the colors of the solutions to be measured, are used, the colorimetric method is nearly as satisfactory as the spectrophotometric method and is much more readily available. Different lots of the dyes used in preparing the artificial color standards may vary in purity and color, which makes it necessary to evaluate each new lot as described above. However, 10 grams of each dye should supply the needs of one worker for several years.

After solutions of the pigments were separated from the green leaves for comparison with the color standards, the volume was increased until accurate readings could be made on the colorimeter. Five readings were made for each determination and the results averaged.

Experimental data

Data on the leaf areas, dry weight of tops, and the dry weight of tops per 100 square cm. of leaf area for all 3 series are given in tables II, III, and IV. In each series, plants of Nebraska 10 had smaller leaf areas and dry weights than those of Nebraska 12, and plants of Nebraska 12 were in turn smaller and lighter than those of Schmidt's White Cap or the F_1 crosses. It will be noted, however, that averaged dry weight per 100 square cm. of leaf area was lowest for plants of Nebraska 12, and that the leaves of Nebraska 10 were about equal to those of Schmidt's White Cap and the F_1 crosses in this respect.

Table IV also brings out the interesting fact that the length of time which leaves remain green is an important factor in determining yield at maturity. Nebraska 10 had a smaller leaf area and dry weight than Nebraska 12 during the first 49 days of growth, but at 96 days it had an equal leaf area. The lower leaves of Nebraska 10 remained green much

TABLE II
LEAF AREAS AND DRY WEIGHTS OF TOPS OF CORN¹ GROWN IN THE GREENHOUSE (1925) IN SAND CULTURES WITH SHIVE'S R₅C₁ NUTRIENT SOLUTION (SERIES 1)

DATE OF HARVEST	AGE OF PLANTS	LEAF AREA, AVERAGE PER PLANT			DRY WEIGHTS OF TOPS, AVERAGE PER PLANT			AVERAGE DRY WEIGHT PER 100 SQ. CM. OF LEAF		
		NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP
(1)	(2)	sq. cm.	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
	days		sq. cm.	sq. cm.	gm.	gm.	gm.	gm.	gm.	gm.
March 14	10	25.4	27.4	13.1						
March 21	17	63.0	90.2	108.1	0.185	0.236	0.266	0.158	0.134	0.128
March 28.	24	144.7	221.3	201.9						
April 4	31	248.8	365.0	530.1						
April 11	38	400.9	558.5	968.8	1.962	2.747	6.031	0.258	0.233	0.305
April 18	45	684.6	840.3	1616.2	3.870	4.829	10.973	0.295	0.263	0.305
April 25	52	1017.5	1415.9	2401.8	6.417	8.371	19.625	0.317	0.268	0.377

¹ 12 plants were averaged in each case to get data on Nebraska 10 and Nebraska 12. The number of plants of Schmidt's White Cap used varied from 2 to 8; and they were 5 days younger throughout the series than indicated in Column 2.

TABLE III

LEAF AREA AND DRY WEIGHT OF TOPS OF CORN¹ GROWN IN THE GREENHOUSE, 1926, IN SAND CULTURES WITH A MODIFIED TOTTINGHAM SOLUTION $T_1B_1C_1$ (SERIES 2)

DATE OF HARVEST	AGE OF PLANTS	LEAF AREA PER PLANT		DRY WEIGHT OF TOPS PER PLANT			DRY WEIGHT PER 100 SQ. CM. OF LEAF		
		NEBRASKA NO. 10	NEBRASKA NO. 12	F_1 ²	NEBRASKA NO. 10	NEBRASKA NO. 12	NEBRASKA NO. 10	NEBRASKA NO. 12	F_1
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	<i>days</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
April 9	10	19.4	29.3	37.4	0.061	0.094	0.122	0.189	0.175
April 16	17	63.5	117.6	144.1	0.236	0.397	0.514	0.217	0.195
April 24	25	188.1	295.1	475.1	0.647	0.970	1.821	0.211	0.179
April 30	31	308.2	604.7	899.1	1.60	3.32	5.27	0.293	0.273
May 7	38	621.6	1203.0	1495.4	3.80	7.94	11.04	0.321	0.300
May 14	45	1233.7	1673.5	2179.4	8.81	14.44	22.83	0.357	0.348

¹ Six plants were averaged in each case to obtain data.

² The F_1 is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE IV
LEAF AREA AND DRY WEIGHTS OF TOPS OF CORN¹ GROWN IN THE FIELD DURING THE SUMMER OF 1925 (SERIES 3)

DATE OF HARVEST	AGE OF PLANTS	AVERAGE LEAF AREA PER PLANT		AVERAGE DRY WEIGHT OF TOPS PER PLANT		AVERAGE WEIGHT PER 100 SQ. CM. OF LEAF		
		NEBRASKA NO. 10	NEBRASKA NO. 12	F ₁	NEBRASKA NO. 10	NEBRASKA NO. 12	NEBRASKA NO. 10	F ₁
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
	<i>days</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>sq. cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
June 9 . . .	10	41.7	66.1	97.2	0.14	0.21	0.32	0.209
" 13 . . .	14	73.8	109.2	198.1	0.28	0.42	0.90	0.236
" 20 . . .	21	167.7	247.6	563.8	0.72	1.03	2.97	0.250
" 27 . . .	28	452.6	740.4	1559.8	2.58	4.44	11.65	0.337
July 4 . . .	35	840.2	1383.2	2773.7	5.70	7.90	18.43	0.363
" 11 . . .	42	2091.1	2934.9	5164.8	16.67	21.47	49.85	0.439
" 18 . . .	49	2943.7	4374.9	6650.9	34.10	51.70	94.65	0.507
Sept. 3 ² . . .	96	3859.6	3858.9	5698.3	Total dry weight at maturity 204.9	166.9	328.7	0.462
					Dry weight of shelled grain 90.1	79.2	182.1	
					Date of maturity Sept. 8	Sept. 4	Sept. 7	

¹ The F₁ is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

² Leaf areas taken on September 3. Plants actually harvested September 15.

TABLE V
 RATES¹ OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN GROWN IN THE GREENHOUSE, 1925, IN SAND CULTURES (SERIES 1)

PERIOD	DAILY RATES OF INCREASE							DAILY AT-MOMETER LOSS	MEAN DAILY TEMPERATURE FOR THE PERIOD	AVERAGE RELATIVE HUMIDITY AT NOON
	LEAF AREA		DRY WEIGHT OF TOPS							
	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP	NEBRASKA NO. 10	NEBRASKA NO. 12	SCHMIDT'S WHITE CAP				
(1)	(2)	(4)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	cc.	degrees C.	per cent.	
March 4-21										
March 22-April 11	8.74	8.68	10.44	11.25	11.45	14.86	24.8	23.4	56	
April 12-18	7.66	5.83	7.31	9.70	8.06	8.55	23.7	21.3	48	
April 19-25	5.64	7.45	5.64	7.23	7.86	8.32	17.7	20.0	53	
Average	7.35	7.32	7.80	9.39	9.12	10.58	18.3	20.4	59	

¹ Daily rates of increase (r), in per cent., were calculated according to the BLACKMAN (2) formula, $\frac{\log W_1 - \log W_0}{t_2 - t_1} = r$, where W_0 and W_1 represent the dry weight of the plants at the beginning and end, respectively, of the experimental interval, $t_2 - t_1$, the time between each two successive harvests.

TABLE VI
 RATES OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN¹ GROWN IN THE GREENHOUSE, 1926, IN SAND CULTURES (SERIES 2)

PERIOD	DAILY RATE OF INCREASE IN LEAF AREA				DAILY RATE OF INCREASE IN DRY WT. OF TOPS			DAILY ATMOMETER LOSS	AVERAGE MEAN DAILY TEMPERA- TURES	RELATIVE HUMIDITY AT NOON
	NEBRASKA NO. 10	NEBRASKA NO. 12	F ₁	per cent.	NEBRASKA NO. 10	NEBRASKA NO. 12	F ₁			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
March 31-April 9...	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	cc.	degrees C.	per cent.	
April 10-16	16.93	19.84	19.26	19.33	20.57	20.55	23.2	21.5	51	
April 17-24	13.57	11.50	14.91	12.60	11.16	15.81	27.8	22.4	32	
April 25-30	8.25	11.96	10.63	15.07	20.49	17.70	29.1	23.0	38	
May 1-7	10.04	9.83	7.27	12.36	12.45	9.86	19.8	20.5	47	
May 8-14	9.78	3.28	5.41	12.07	8.56	10.37	27.4	22.5	38	
Average	11.71	11.28	11.50	14.29	14.65	14.86	30.3	22.1	40	

¹ The F₁ is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE VII
RATES OF GROWTH AND ATMOSPHERIC CONDITIONS FOR CORN¹ GROWN IN THE FIELD, 1925 (SERIES 3)

PERIOD	DAILY RATE OF INCREASE IN LEAF AREA			DAILY RATE OF INCREASE IN DRY WEIGHT OF TOPS			TOTAL ² RAINFALL FOR THE PERIOD	MEAN DAILY TEMPERATURE FOR THE PERIOD
	NEBRASKA NO. 10	NEBRASKA NO. 12	F ₁	NEBRASKA NO. 10	NEBRASKA NO. 12	F ₁		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
June 1-9	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	inches	degrees C.
June 10-13	14.27	12.52	17.80	17.33	17.33	25.83	0.00	26.7
June 14-20	11.71	11.68	14.94	13.48	12.80	17.06	0.00	20.6
June 21-27	14.19	15.65	14.54	18.23	20.87	19.52	0.14	23.9
June 28-July 4	8.93	8.94	8.24	11.33	8.24	6.54	0.10	22.2
July 5-11	13.03	10.73	8.86	15.34	14.30	14.21	1.78	21.7
July 12-18	4.91	5.70	3.60	8.80	12.57	9.17	1.03	25.0
Average	11.17	10.87	11.33	14.08	14.35	15.39	0.48	23.3

¹ The F₁ is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

² 0.68 inches precipitation for last three days of May.

longer than those of Nebraska 12 or the F_1 as the plants approached maturity, which may account in part for the greater final yield of tops and grain from Nebraska 10 than from Nebraska 12. In all 3 series, the differences in dry weights of the strains were more marked than the differences in leaf areas, thus indicating that the leaves of the different strains were probably functioning with different degrees of efficiency in the assimilation of carbon.

GROWTH RATES

Data on the rates of growth and the atmospheric conditions for the 3 series are given in tables V, VI, and VII. The daily rates of increase in leaf area and in dry weight of tops were calculated according to BLACKMAN's formula (2). In general, the daily rates of increase in leaf area varied in the same direction as daily rates of increase in dry weight of tops but the rates were not proportional nor equal.

In series 1, there was a steady decline in growth rates with the age of the plant in all three strains, which is in accord with the findings of other workers studying growth rates. In series 2 and 3, the general decline in growth rates, although apparent, was by no means steady, particularly for dry weight of tops. In series 2, the most notable deviation in daily rates of increase in dry weight of tops was for the period April 25-30 when the rates of all strains were higher than for the preceding period. Since this deviation was accompanied by a marked increase in relative humidity of the air, the change in humidity may be taken as the cause.

The daily rates of increase in dry weights of tops for series 3, were abnormally low during the periods June 14-20, and June 28-July 4. The depression in the first period may be attributed to the absence of rain from May 30 to June 16. During the latter part of the period soil moisture had been so depleted by evaporation and transpiration that it constituted a limiting factor in growth. Light rains coming between June 16 and 25 caused an increase in growth, but the precipitation was not sufficient to maintain growth rates in the June 28-July 4 period. After July 1, rainfall was adequate for normal growth.

It may be noted in each series that growth rates, as measured by increase in dry weight of tops, were not paralleled by corresponding increases in leaf area. The conditions which caused changes in the growth rates based on dry weight, apparently operated by modifying the efficiency with which a given area of leaf functioned, as well as by changing the total leaf area.

Moreover, the efficiency with which the leaves of the various strains functioned was apparently not equal. In general, the rates of increase of leaf area were greater for Nebraska 10 than for Nebraska 12, but the rates

TABLE VIII

PIGMENT FORMATION IN CORN GROWN IN SAND CULTURES IN THE GREENHOUSE, 1925, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 1)

AGE OF PLANTS	STRAIN USED	TOTAL LEAF AREA EX- TRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF ¹						CALCULATED DRY WEIGHT OF FRESH LEAVES EXTRACTED	AMOUNT OF PIGMENT PER GRAM OF LEAF (DRY WEIGHT) EXTRACTED ¹					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
days		sq. cm.	mg.		mg.		mg.		gm.	mg.		mg.		mg.	
17	Nebraska 10	284.8	2.61	100	0.038	100	0.018	100	0.228	22.7	100	0.335	100	0.158	100
17	Nebraska 12	340.0	1.57	60	0.048	124	0.012	67	0.308	31.3	138	0.527	157	0.140	89
12	Schmidt's														
*	White Cap	67.7	3.07	118	0.066	174	0.009	50	0.077	30.2	133	0.576	172	0.080	51
38	Nebraska 10	1035.6	4.06	156	0.125	329	0.112	622	2.196	19.2	84	0.590	176	0.529	335
38	Nebraska 12	1671.6	3.60	138	0.121	318	0.138	767	3.116	19.3	85	0.650	194	0.746	472
33	Schmidt's														
	White Cap	386.7	2.46	94	0.163	429	0.244	1356	0.962	14.9	66	0.660	197	0.982	692
45	Nebraska 10	1644.4	3.07	118	0.104	274	0.108	600	4.844	10.4	46	0.348	104	0.366	232
45	Nebraska 12	2448.0	2.46	94	0.164	432	0.097	539	6.444	9.3	41	0.403	120	0.371	235
40	Schmidt's														
	White Cap	688.1	2.87	110	0.119	313	0.054	300	2.096	9.4	41	0.391	117	0.181	115
Average	Nebraska 10		3.25		0.089		0.079			17.4		0.424		0.351	
	Nebraska 12		2.54		0.111		0.082			20.0		0.528		0.419	
	Schmidt's														
	White Cap		2.80		0.116		0.102			18.2		0.542		0.414	

¹ Relative values are calculated using Nebraska 10 at 17 days as 100.

TABLE IX

PIGMENT FORMATION IN CORN GROWN IN SAND CULTURES IN THE GREENHOUSE, 1926, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 2)

AGE OF PLANTS	STRAIN USED ¹	TOTAL LEAF AREA EX- TRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF ²						CALCU- LATED DRY WEIGHT OF FRESH LEAVES EX- TRACTED	AMOUNT OF PIGMENT PER GRAM (DRY WEIGHT) OF LEAF EXTRACTEDS					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELA- TIVE	ACTUAL	RELA- TIVE	ACTUAL	RELA- TIVE		ACTUAL	RELA- TIVE	ACTUAL	RELA- TIVE	ACTUAL	RELA- TIVE
(1) days	(2)	(3) sq. cm.	(4) mg.	(5)	(6) mg.	(7)	(8) mg.	(9)	(10) gm.	(11) mg.	(12)	(13) mg.	(14)	(15) mg.	(16)
10	Nebraska 10	61.5	1.75	100	0.093	100	0.085	100	0.116	9.2	100	0.490	100	0.450	100
10	Nebraska 12	67.6	1.90	109	0.100	108	0.095	112	0.118	10.9	118	0.576	118	0.547	122
10	F ₁	78.3	2.06	118	0.121	130	0.118	139	0.146	11.0	120	0.646	132	0.632	140
17	Nebraska 10	128.2	2.32	133	0.112	120	0.134	158	0.276	10.8	117	0.520	106	0.624	139
17	Nebraska 12	162.7	2.71	155	0.140	151	0.115	135	0.316	14.0	152	0.718	147	0.593	132
17	F ₁	183.7	2.41	138	0.123	132	0.118	139	0.388	11.4	124	0.584	119	0.563	125
25	Nebraska 10	207.7	2.39	137	0.100	108	0.112	132	0.438	11.3	123	0.474	97	0.534	119
25	Nebraska 12	460.5	2.60	149	0.106	114	0.062	73	0.822	14.6	159	0.593	121	0.344	76
25	F ₁	610.7	2.90	166	0.125	134	0.089	105	1.318	13.4	146	0.573	117	0.415	92
31	Nebraska 10	445.0	2.27	130	0.091	98	0.115	135	1.31	7.7	84	0.306	62	0.392	87
31	Nebraska 12	853.4	2.00	114	0.108	116	0.132	155	2.32	7.4	80	0.395	81	0.486	108
31	F ₁	1126.0	2.72	155	0.132	142	0.138	162	3.31	9.2	100	0.452	92	0.470	104
38	Nebraska 10	655.0	2.66	152	0.106	114	0.161	189	2.13	8.2	89	0.323	66	0.498	111
38	Nebraska 12	1342.6	2.99	171	0.130	140	0.134	158	4.00	10.3	112	0.435	89	0.447	99
38	F ₁	1417.2	2.89	165	0.134	144	0.143	168	4.72	8.6	93	0.404	82	0.427	95
45	Nebraska 10	1249.4	4.41	252	0.134	144	0.120	141	4.45	12.4	135	0.376	77	0.338	75
45	Nebraska 12	1926.8	5.58	319	0.164	176	0.101	119	6.71	16.0	174	0.472	96	0.291	65
45	F ₁	2318.0	6.68	382	0.174	187	0.112	132	9.07	17.1	186	0.446	91	0.286	64
Average	Nebraska 10		2.63		0.106		0.121			9.9		0.415		0.473	
	Nebraska 12		2.96		0.125		0.106			12.2		0.531		0.451	
	F ₁		3.28		0.135		0.120			11.8		0.517		0.465	

¹ The F₁ is a cross between Nebraska 12 (♀) and Nebraska 10 (♂).² Relative values are calculated using Nebraska 10 at 10 days as 100.

TABLE X

PIGMENT FORMATION IN CORN GROWN IN THE FIELD, 1925, WITH RELATION TO THE AREA AND DRY WEIGHT OF THE LEAVES (SERIES 3).

AGE OF PLANTS	STRAIN USED ¹	TOTAL LEAF AREA EX- TRACTED	AMOUNT OF PIGMENT PER 100 SQ. CM. OF LEAF ²						CALCULATED DRY WEIGHT OF FRESH LEAVES EX- TRACTED	AMOUNT OF PIGMENT PER GRAM (DRY WEIGHT) OF LEAF EXTRACTED ³					
			CHLOROPHYLL		CAROTIN		XANTHOPHYLL			CHLOROPHYLL		CAROTIN		XANTHOPHYLL	
			ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE
(1) days	(2)	(3) sq. cm.	(4)	(5)	(6) mg.	(7)	(8)	(9)	(10) gm.	(11)	(12)	(13) mg.	(14)	(15) mg.	(16)
10	Nebraska 10	68.2	7.44	187	0.665	652	1.100	853	0.142	35.7	300	3.194	1044	5.284	1369
10	Nebraska 12	139.2	4.69	118	0.181	177	0.387	300	0.274	23.8	200	0.924	302	1.963	509
10	F ₁	158.6	6.42	161	0.112	110	0.380	295	0.328	31.1	261	0.542	177	1.937	476
14	Nebraska 10	136.4	4.99	125	0.189	185	0.378	293	0.322	21.1	177	0.798	261	1.604	416
14	Nebraska 12	174.8	5.90	148	0.100	98	0.869	674	0.402	25.7	216	0.433	142	3.779	979
14	F ₁	338.8	5.40	136	0.019	19	0.028	22	0.960	19.0	160	0.066	22	0.100	26
21	Nebraska 10	171.2	0.047	46	0.209	162	0.438	0.181	59	0.815	211
21	Nebraska 12	378.0	3.67	92	0.083	81	0.297	230	0.898	15.5	130	0.350	114	1.247	323
21	F ₁	737.6	5.17	130	0.129	126	0.169	131	2.296	16.6	140	0.410	134	0.541	140
28	Nebraska 10	315.8	3.98	100	0.102	100	0.129	100	1.060	11.9	100	0.306	100	0.386	100
28	Nebraska 12	540.0	4.54	114	0.132	129	0.149	116	1.650	14.9	125	0.433	142	0.486	126
28	F ₁	211.5	7.27	183	0.219	214	0.154	119	0.790	19.5	164	0.586	192	0.410	106
35	Nebraska 10	546.6	5.78	145	0.115	113	0.143	111	1.960	16.1	135	0.321	105	0.398	103
35	Nebraska 12	826.8	5.44	137	0.132	129	0.149	116	2.580	17.4	146	0.425	139	0.480	124
35	F ₁	1441.0	5.86	147	0.136	133	0.109	84	5.270	16.0	134	0.370	121	0.300	78
42	Nebraska 10	1164.4	4.60	116	0.138	135	0.181	140	5.090	10.5	88	0.318	104	0.417	108
42	Nebraska 12	1484.8	3.61	91	0.153	150	0.160	124	5.350	10.0	84	0.423	138	0.444	115
42	F ₁	2214.6	4.03	101	0.204	200	0.148	115	9.320	9.6	81	0.486	159	0.352	91
49	Nebraska 10	1493.8	4.27	107	0.183	179	0.211	164	7.560	8.4	71	0.361	118	0.417	108
49	Nebraska 12	2064.0	4.90	123	0.193	189	0.169	131	9.750	10.4	87	0.408	133	0.360	93
49	F ₁	2682.4	5.62	141	0.215	211	0.175	136	13.610	11.1	93	0.423	138	0.346	90
Average	Nebraska 10		4.66		0.134		0.166			11.7		0.326		0.404	
last 4	Nebraska 12		4.62		0.152		0.157			13.2		0.422		0.440	
harvests	F ₁		5.69		0.193		0.146			14.0		0.466		0.352	

¹ The F₁ is a cross between Nebraska 12 (♀) and Nebraska 659 (♂).² Relative values are calculated using Nebraska 10 at 28 days as 100.

of increase in dry weight averaged higher for Nebraska 12 than Nebraska 10. Also, the average rates of increase in dry weight of the F_1 crosses were higher than those of Nebraska 10 in series 2 and 3, although the average rates of increase in leaf area were about the same.

CHLOROPLAST PIGMENT CONTENT PER UNIT OF LEAF

Data on the pigment content of each strain per unit of leaf area, and of leaf weight, at successive harvests of the 3 series are given in tables VIII, IX, and X. It has been noted that the leaves of the various strains apparently functioned with varying efficiencies which could not be explained on the basis of differences in leaf area. Since chlorophyll is known to be a primary agent in the synthesis of plant food, it is possible that the variation in concentration of this pigment is a contributing factor in the rate of carbon assimilation, and therefore in growth. The chloroplast pigment concentrations have been expressed both on the basis of unit of leaf area, and of unit of dry weight, because the two criteria give quite different values.

In series 1, on the basis of milligrams of pigment per 100 square cm. of leaf, there was no distinct trend noted in chlorophyll concentration at successive harvests; also, the relation between these concentrations and the growth rates of a single strain was not consistent. On the basis of chlorophyll concentration per gram of leaf, the highest values for each strain were found in the earlier harvest, with later harvests showing declining values. These declining values were associated with increases in weight of leaf per unit of area at successive harvests. The inverse relation between chlorophyll content per gram of leaf and age of the plant was also observed in series 3. Since there was no decline noted in chlorophyll concentration per unit of leaf area, it seems clear that this pigment was much more closely correlated with the area of leaves than with their dry weight. Unlike the other series, the plants of series 2 showed no consistent decline in chlorophyll per gram of leaf at the successive harvests. This discrepancy is unintelligible at this time because too little is known about the effects of environmental conditions on pigment formation.

There was only one period, occurring in series 3, when there seemed to be a close correlation between growth rates and the chlorophyll concentrations of all strains. The concentrations of the pigment on either the dry weight or leaf area basis, were somewhat higher at the beginning of the period July 5-11 than for the preceding one. This increase in pigment concentration was accompanied by an improvement in the supply of soil moisture and appears to have been caused by it.

In series 1, it may be noted that correlation between concentrations of chlorophyll and increase in dry weight of the various strains was fairly good. Nebraska 12 had the lowest concentration per unit of area and the lowest

growth rate. Nebraska 10 was intermediate in concentration of chlorophyll and in growth, while Schmidt's White Cap has the highest average values for both characters.

The data from series 2 are of the most value in showing the relation between chlorophyll concentration of the strains and the rates of increase in dry weight of their tops, because the environmental conditions were more nearly uniform throughout the entire period than for the other two series. Nebraska 10, which had the lowest average rate of growth, showed the lowest values of chlorophyll per unit of leaf area and per unit of dry weight in five out of six harvests. Nebraska 12 which was intermediate in the average increase in dry weight of tops, had intermediate values of chlorophyll per unit of leaf area four times out of six, and on the basis of unit leaf weight showed intermediate values twice, the highest values three times, and the lowest once. The F_1 plants of this series which had the highest average rate of increase in dry weight of tops also had the highest concentration of chlorophyll per unit of area five times out of six, and had the highest values per gram of leaf three times, intermediate values twice, and lowest value once.

The chlorophyll concentrations per unit area of leaf in series 3 were somewhat erratic for the first three harvests, probably because of high temperatures and lack of rainfall. With these harvests eliminated, the last four harvests showed a fairly close relation between chlorophyll content of the various strains and the average rates of increase in dry weight of tops. Nebraska 10, with the lowest average growth rate, had the lowest set of concentration values for chlorophyll, Nebraska 12 showed intermediate values in both rate of growth and chlorophyll concentration, and the F_1 had the highest values for both characters.

A significant feature of the chlorophyll concentrations per unit of leaf area was the relatively high values observed in all three strains of series 3, as compared with the first two series. The explanation may lie in the light relations of the three series. The plants of series 3 were grown during a season when the days were longer and the light more intense than those of series 1 and 2. Also, being grown in the field, no portion of the spectrum was excluded or diminished in intensity by glass. Unfortunately no exact measures of light conditions were made in connection with these experiments.

When the concentrations of carotin and chlorophyll are compared for all three series, it becomes apparent that there was a fairly close correlation between the concentrations of the two pigments. The correlations were somewhat higher on the basis of unit dry weight than on the basis of unit leaf area. Apparently, conditions which affect the concentration of one pigment also affect that of the other in like manner. The most notable

exception to this condition occurred at the first two harvests of series 3. Carotin values were very high, especially on the dry weight basis, and their abnormal values were not accompanied by similar values of chlorophyll. These periods were characterized by unusually high temperatures and lack of rainfall. The different strains did not behave alike under these conditions; Nebraska 10 had a much higher concentration of carotin than Nebraska 12, while the F_1 values were apparently quite normal. These variations did not seem to be significantly related to the growth rates for the periods following the analyses.

With the exception of series 1, the relation between rates of increase in dry weight of tops and concentrations of carotin was even closer than the relation between the growth rates and chlorophyll concentration of the various strains. In series 1, Nebraska 10 averaged lower concentrations of carotin on both the dry weight and leaf area bases than did Nebraska 12 but had slightly higher average growth rates. The concentrations of carotin in Schmidt's White Cap were greater than those of either Nebraska 10 or Nebraska 12 and these were accompanied by higher growth rates.

In series 2, the correlation between concentrations of carotin per unit of leaf area and the rates of growth of the various strains was very good. Nebraska 10 had the lowest set of concentrations and the lowest average growth rate, Nebraska 12 gave intermediate values and the F_1 the highest values. On the basis of carotin concentration per gram of leaf, Nebraska 10 again had the lowest average values for both pigment concentration and growth rate, but Nebraska 12 had a higher pigment concentration than the F_1 and a lower average rate of growth.

Disregarding the few abnormal data of the first harvests of series 3, there was a very good correlation between the average carotin concentration of the three strains and their average growth rates. The relation was slightly closer on the basis of unit leaf area than on the basis of unit dry weight; but Nebraska 10 had the lowest average concentrations of carotin and also the lowest average calculated on either basis; Nebraska 12 gave intermediate concentrations, and the F_1 gave highest.

The concentrations of xanthophyll fluctuated so widely in these series that it is difficult to detect any distinct trends or relationships. The concentrations of xanthophyll in plants of Nebraska 10 were not consistently higher or lower than those of the Nebraska 12 plants on either the basis of unit leaf area or of unit dry weight. Xanthophyll concentrations resembled carotin concentrations only during the first two harvests of series 3 when high concentrations of both pigments occurred in conjunction with high temperatures and a scanty moisture supply.

Neither carotin nor xanthophyll exhibited any decline in concentration per gram of leaf with successive harvests, such as was observed with chloro-

TABLE XI
TOTAL CHLOROPLAST PIGMENTS FOUND IN THE LEAF BLADES OF CORN PLANTS GROWN IN SAND CULTURES IN THE GREENHOUSE, 1925 (SERIES 1)

AGE OF PLANTS	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10		SCHMIDT'S WHITE CAP ¹	NEBRASKA 10		NEBRASKA 12	NEBRASKA 10		SCHMIDT'S WHITE CAP
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1)									
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
17	1.64	1.42	3.32	0.024	0.042	0.071	0.011	0.011	0.010
38	16.28	20.11	23.83	0.501	0.676	1.579	0.449	0.771	2.364
45	21.02	20.67	46.38	0.712	1.378	1.923	0.739	0.815	0.873

¹ Schmidt's White Cap was 5 days younger throughout the series than indicated.

TABLE XII
TOTAL CHLOROPLAST PIGMENTS FOUND IN LEAF BLADES OF CORN PLANTS GROWN IN THE GREENHOUSE IN 1926 (SERIES 2)

AGE OF PLANT	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10	NEBRASKA 12	F ₁ ¹	NEBRASKA 10	NEBRASKA 12	F ₁ ¹	NEBRASKA 10	NEBRASKA 12	F ₁ ¹
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10	0.34	0.56	0.77	0.018	0.029	0.045	0.016	0.028	0.044
17	1.47	3.19	3.47	0.071	0.165	0.177	0.085	0.135	0.170
25	4.50	7.67	13.78	0.188	0.313	0.594	0.211	0.183	0.423
31	7.00	12.09	24.46	0.281	0.653	1.187	0.354	0.798	1.241
38	16.54	35.97	43.22	0.659	1.564	2.004	1.001	1.612	2.138
45	54.41	93.38	145.58	1.653	2.744	3.792	1.480	1.690	2.441

¹ The F₁ in this series was a cross between Nebraska 12 (♀) and Nebraska 10 (♂).

TABLE XIII

TOTAL CHLOROPLAST PIGMENTS FOUND IN THE LEAF BLADES OF CORN PLANTS GROWN IN THE FIELD DURING THE SUMMER OF 1925 (SERIES 3)

AGE OF PLANT	TOTAL CHLOROPHYLL, AVERAGE PER PLANT			TOTAL CAROTIN, AVERAGE PER PLANT			TOTAL XANTHOPHYLL, AVERAGE PER PLANT		
	NEBRASKA 10		F ₁ ¹	NEBRASKA 10		F ₁ ¹	NEBRASKA 10		F ₁ ¹
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
days	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10	3.10	3.10	6.24	0.277	0.120	0.109	0.459	0.256	0.369
14	3.68	6.44	10.70	0.139	0.109	0.038	0.279	0.949	0.065
21	...	9.09	29.15	0.079	0.206	0.727	0.350	0.735	0.953
28	18.01	33.61	113.40	0.462	0.977	3.416	0.584	1.103	2.402
35	48.56	75.25	162.54	0.966	1.826	3.772	1.201	2.061	3.023
42	96.19	105.95	208.14	2.886	4.490	10.536	3.785	4.696	7.644
49	125.70	214.37	373.78	5.387	8.444	14.299	6.211	7.394	11.639

¹ The F₁ in this series was a cross between Nebraska 12 (♀) and Nebraska 659 (♂).

phyll. On the basis of unit leaf areas, there was a slight increase in concentrations of carotin with successive harvests in all three series, provided the first two harvests of series 3 are disregarded. These data indicate that the formation of the carotinoids was closely related to the weight of the leaves, whereas the formation of chlorophyll was closely related to their surface area. Evidently carotin and xanthophyll have somewhat similar modes of formation and perhaps have similar physiological functions.

TOTAL CHLOROPLAST PIGMENT CONTENT AND YIELDS OF DRY MATTER

The total chloroplast pigment content in the leaf blades of a plant is dependent upon the concentration of pigment per unit of area and the total area of the leaves. If the chloroplast pigments are primary agents in the synthesis of organic compounds in the plant, there should be a fairly close correlation between the total amount of these pigments present and the total dry weight produced. The data presented above indicate only the quantities of chloroplast pigments found in the leaf blades, ignoring that small fraction which was present in the leaf sheaths and stems; and the data on dry weights do not include roots. If correlations between quantities of chloroplast pigments in leaf blades and dry weight of tops are high, similar correlations may reasonably be expected between the total amount of such pigments in the whole plant and total dry weight, providing the ratios of tops to roots are similar for all strains. The total quantity of chloroplast pigments found in the leaf blades of the strains in these experiments is given in tables XI, XII, and XIII.

There was a well-defined correlation between the total chlorophyll content of the various strains and their respective dry weight of tops. (Compare tables II, III and IV with tables XI, XII and XIII.) Nebraska 10 had the smallest quantity of chlorophyll per plant at every harvest of series 2 and 3, and also had the smallest dry weight of tops at all times. Nebraska 12 had larger quantities of chlorophyll and greater dry weights of tops than Nebraska 10 for every harvest in series 2 and 3, but less than Schmidt's White Cap in series 1 and the F_1 crosses in series 2 and 3.

The correlation between the total carotin content of leaves of the various strains and their total dry weight of tops was nearly as close as that noted for chlorophyll, with the exception of the first two harvests of series 3. In series 2 and 3, Nebraska 10 had the lowest carotin content and the lowest dry weight of tops; and Nebraska 12 had higher values for both characters than Nebraska 10, but lower values than the F_1 crosses.

Total xanthophyll values were also closely correlated with dry weight of tops, but not as closely as carotin values. In addition to the unusual values obtained in the first two harvests of series 3, the values for the first and third harvests of series 1 and the third harvest of series 2 did not show

TABLE XIV
THE RELATIONS BETWEEN THE DRY WEIGHT OF CORN PLANTS (TOPS) AND THE TOTAL CONTENT OF CHLOROPLAST PIGMENTS IN THE LEAF BLADES
AS SHOWN BY RATIOS (SERIES 2)

AGE OF PLANTS	RATIO OF DRY WEIGHT TO TOTAL CHLOROPHYLL CONTENT			RATIO OF DRY WEIGHT TO TOTAL CAROTIN CONTENT			RATIO OF DRY WEIGHT TO TOTAL XANTHOPHYLL CONTENT		
	NEBRASKA 10		F ₁	NEBRASKA 10		F ₁	NEBRASKA 10		F ₁
	(2)	(3)		(5)	(6)		(8)	(9)	
(1) <i>days</i>			(4)			(7)			(10)
10	179.4	167.9	158.4	3389	3241	2711	3812	3357	2773
17	160.5	124.5	148.1	3324	2406	2904	2776	2941	3024
25	144.8	126.5	132.1	3441	3099	3066	3066	5301	4305
31	228.6	274.6	215.5	5694	5084	4440	4520	4160	4247
38	229.7	220.7	255.4	5766	5077	5509	3796	4925	5164
45	161.9	154.6	156.8	5330	5262	6021	5953	8544	9353
Average	184.1	178.1	177.7	4491	4028	4108	3987	4871	4811

the same differences between strains as their dry weights. With these exceptions, Nebraska 10 was lowest in total xanthophyll and dry weight of tops, and Nebraska 12 was higher than Nebraska 10 but lower than Schmidt's White Cap and the F_1 crosses.

It is important also to determine the degree of relationship between the quantity of each chloroplast pigment and dry weight of tops. The data are too few to permit the calculation of reliable coefficients of correlation but the relation may be expressed in another fashion. Series 2 is most satisfactory for a study of this sort, since the environmental conditions were more uniform throughout the entire experimental period than for the other series. The relation has been expressed in table XIV in terms of ratios between grams of dry matter and grams of pigment.

The average ratios between dry weight and chlorophyll content were very similar for all three strains of corn. A rise or fall in the ratio of one strain at any harvest was usually accompanied by a similar rise or fall in the other strains. The fluctuations in ratios for the various harvests signify that total chlorophyll content was not the only causal factor involved in growth, but the relatively small size of the fluctuations indicates that it was a very important factor.

The average ratios between dry weight and carotin content were similar for all three strains of corn, but displayed greater differences than those noted for chlorophyll. The differences between ratios of the strains at various harvests were much more marked than those for chlorophyll. In general, however, there was a distinct tendency for fluctuations of all three strains to move in the same direction.

The average ratios (and those for each harvest) between dry weight and xanthophyll content were even less consistent than those noted for carotin. In only two of the six harvests did the ratios of all three strains fluctuate in the same direction.

It seems clear from these data that a close relation existed between total chlorophyll content and dry weight of tops for all three strains of corn, at the various stages of growth considered. It may be assumed that the relation was a causal one since chlorophyll is known to be essential in photosynthesis. The physiological functions of carotin and xanthophyll are not known, and the correlations between quantities of these pigments and dry weights in this series of plants have not demonstrated their essentiality in growth. The data in table XIV indicate that the closest correlation existed between dry weight and chlorophyll, the next in order between dry weight and carotin, and the lowest correlations between dry weight and xanthophyll.

Discussion and conclusions

Although the question of the relations between chloroplast pigments, leaf area, and dry weight has not been definitely defined by the data here

presented, certain points seem fairly clear. As a whole there is a much higher correlation between chlorophyll content and leaf area than between chlorophyll content and dry weight of leaves. On the other hand, data from series 2 and 3 indicate that carotin and xanthophyll are somewhat more closely related to leaf weight than to leaf area.

Chlorophyll concentrations per unit of area, or of dry weight, were not closely paralleled by rates of increase in dry weight of tops from period to period for a single strain, but chlorophyll concentrations of the various strains were rather closely correlated with their respective rates of increase in dry weight. Thus, the strain which had the lowest set of chlorophyll values per unit of leaf area, also had the lowest average rate of increase in dry matter, and *vice versa*. Likewise, the correlations between carotin concentrations and rates of increase in dry matter were fairly close, but the xanthophyll readings were so variable that no definite relation could be observed. These conclusions rest on the assumption that the leaves selected for pigment extraction were representative of all of the leaves on the plant with respect to pigment formation, leaf area and dry weight. Since comparable leaves from each plant of every strain of corn were harvested for each set of determinations, it seems reasonable to suppose that no great error has been introduced.

It has been suggested by some investigators that chlorophyll is being constantly synthesized and broken down in the leaf. If this is the case, the amount present at any one time in the leaf represents the result of an equilibrium between the synthetic and destructive processes. On this basis it is obvious that the chlorophyll content of a leaf or of a plant at any given instant may not be representative of that present at any other time during a weekly period. With constant fluctuations in quantity of chlorophyll as a result of environmental or cultural conditions, there is considerable chance of obtaining values for the pigment which do not represent average conditions and this may partly account for the failure to obtain perfect correlations between chlorophyll concentration and rates of increase in dry weight. The same observation applies also to measurements of carotin and xanthophyll. The data presented have not revealed how environmental or cultural conditions affect the chloroplast pigment content of a plant at any given time.

Internal factors not directly associated with pigment content are undoubtedly contributing factors in dry weight production. With adequate amounts of chlorophyll and carotinoids present, there still may be distinctly different rates of growth in different strains due to the effect of these undetermined internal factors.

Another significant point noted in these experiments was the close correlation between total chloroplast pigment content of the leaves of the

various strains and their respective dry weights. With but two exceptions, occurring in series 1, the strain having the highest chlorophyll content at any stage of development also had the highest dry weight of tops, and *vice versa*. The correlation was particularly clear in series 2 grown under uniform environmental conditions as far as this was possible. Since chlorophyll has been shown to be essential for photosynthesis, and since these data show a high correlation between total chlorophyll content and dry weight, the logical conclusion is that superiority in dry weight of the plants of one strain over those of another strain similarly treated was largely due to superiority in chlorophyll content. Undoubtedly other factors are involved since the ratio between grams of dry matter and grams of chlorophyll was not absolutely constant; but these factors must have been of minor value, or the average ratios of the three strains of corn would not have been so nearly alike.

Since none of these strains could be classed as chlorophyll deficient by observation, it seems likely that chlorophyll tests may become of considerable value in the improvement of corn. If the more vigorous strains may be selected on this basis plus the visual evidences of vigor, a portion of the tedious hybridizing and progeny testing which is now necessary for selection of desirable strains may be eliminated.

Although a fairly close correlation appears to exist between dry weight and total quantities of carotin and xanthophyll, it is not safe to assume that the relation is causal because these pigments have not been shown to be essential for synthesis of organic matter. Their occurrence in amounts rather uniformly proportional to chlorophyll in series 2 suggests a similar relation to dry weight but the wide fluctuations which occurred under field conditions also suggest that the carotinoids may play a different rôle in the life processes of the plant from that usually attributed to chlorophyll.

Summary

Data on leaf area, dry weight of tops and chloroplast pigment content were obtained from three series of corn plants. Two of the series were grown in the greenhouse, using sand cultures with culture solutions added by the constant drip method. The other series was grown in the field. Three strains of corn were grown in each experiment and two of these strains were used in all three series, namely, Nebraska 10 and Nebraska 12, which have been self-pollinated for 17 generations. The other strain used was either a commercial variety or an F_1 cross of two selfed strains. Weekly harvests and determinations of dry weight of tops, leaf area (expanse), and chloroplast pigment content were made. Growth rates were calculated for both dry weight of tops and leaf area, and data on atmospheric conditions for the same periods were obtained. The pigments

were extracted from comparable leaves of the different plants, separated, purified, and made up to a definite volume for colorimetric determinations in a Duboseq colorimeter. Artificial color standards were used in measuring the various pigments, each color standard having been evaluated in terms of milligrams of the pigment to be measured.

1. Rates of increase of leaf area and of dry weight of tops both declined normally as the plant aged, but they were not always closely correlated.

2. The strain or variety having the smallest leaf area during the period of an experiment also produced the lowest dry weight of top, and *vice versa*.

3. Chloroplast pigment measurements failed to show a proportional relation to the measured growth rates of a given strain, no matter whether expressed on the basis of pigment per 100 sq. cm. of leaf area or per gram of leaf (dry weight).

4. The strain which showed high chlorophyll and carotin concentrations per unit of leaf area also had high average rates of increase in dry weight of tops, and *vice versa*. Xanthophyll values were variable and showed no definite relation to rates of growth.

5. Chlorophyll was more closely correlated with leaf area than with dry weight of leaves. Carotin and xanthophyll, however, were not as closely correlated with leaf as with dry weight of leaves.

6. The dry weights per unit of leaf area increased for all strains of corn at successive harvests. Nebraska 12 leaves had the lowest weight per 100 sq. cm. of leaf area in all three series, Nebraska 10 next lowest, and the commercial variety or F₁ cross, the highest weight.

7. In two of the three series of plants grown, the weight of chlorophyll per gram of leaf (dry weight) decreased as the leaves increased in weight per 100 sq. cm. of area. In the other series no clear relation could be observed in this respect.

8. There was a close correlation between the total quantity of chlorophyll contained in the leaves of the various strains of maize and their dry weight of tops at successive harvests. Correlations between total carotin content and dry weight, and between total xanthophyll content and dry weight were close enough to be significant but were not as exact as those noted between total chlorophyll content and dry weight.

9. The average ratios between total chlorophyll and dry weight of tops were practically identical with all three strains of corn in series 2. Ratios between dry weight and carotin content of the same series were more variable than those noted between dry weight and chlorophyll but a certain constancy was apparent. Ratios between dry weight and xanthophyll are so variable that their significance is doubtful.

LITERATURE CITED

1. ALLISON, R. V. The relation of aeration to the development of the soybean plant in artificial culture. New Jersey Agr. Exp. Sta. Ann. Rpt. pp. 338-344. 1921.
2. BLACKMAN, V. H. The compound interest law and plant growth. Ann. Bot. **33**: 353-360. 1919.
3. JONES, L. H., and SHIVE, J. W. Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulfate as sources of iron. Jour. Agr. Res. **21**: 701-728. 1921.
4. MONTGOMERY, E. G. Correlation studies in corn. Nebraska Agr. Exp. Sta. 24th Ann. Rpt. 113, 114. 1911.
5. SCHERTZ, F. M. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **26**: 383-400. 1923.
6. ————. The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. Jour. Agr. Res. **30**: 253-261. 1925.
7. ————. The extraction and separation of chlorophyll (α and β), carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Phys. **3**: 211-216. 1928.
8. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 373-397. 1915.
9. ————, and STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**. 317-323. 1927.
10. SPOEHR, H. A. Photosynthesis. New York. 1926.
11. SPRAGUE, H. B. Preliminary report on the relations between pigment formation, leaf area, and dry weight of corn grown in sand cultures. Ann. Rpt. New Jersey Agr. Exp. Sta. for the year ending June 30, 1925. pp. 332-337. 1925.
12. STILES, WALTER. Photosynthesis. The assimilation of carbon by green plants. London. 1925.
13. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133-245. 1914.
14. WILLSTÄTTER, R., and STOLL, A. Untersuchungen über Chlorophyll. Berlin. 1913.
15. ————, ————. Untersuchungen über die Kohlensäure-assimilation. Berlin. 1918.

THE TOXIC SUBSTANCE PRODUCED BY THE EYE-SPOT FUNGUS OF SUGAR CANE, *HELMINTHOSPORIUM SACCHARI* BUTLER

ATHERTON LEE

(WITH TWO FIGURES)

Introduction

Eye spot is the name given to a leaf disease of sugar cane caused by the fungus *Helminthosporium* (*Cercospora*) *sacchari* Butler.¹ The disease is characterized by small spots on the leaves of most varieties, but on more susceptible varieties long streaks, several feet in length and sometimes 5/16ths of an inch in width, may result from infection. When there are many infections per leaf on such susceptible varieties, whole leaves may die, and with an inadequate leaf supply the growing point of the cane frequently dies also; the whole cane stalk then may rot from secondary infections or in other ways be useless for milling. In case the environmental conditions are very favorable for the disease, from 20 to 80 per cent. of the stalks may be killed in this manner, resulting in severe financial loss.

It has been carefully shown by LEE and MARTIN (3) and MARTIN and LEE (4) that nitrogen fertilizers greatly increase the severity of this disease. This increased severity was at first thought to result merely from more succulent, actively growing host tissues which resulted from the fertilizer applications, since it is usually found that eye spot is more severe on the young, new, actively growing leaves, while more slowly growing hardened leaves are not severely affected. However, as against this theory was the finding by LEE and MARTIN (3), that increased cane growth as a response to phosphoric acid, did not increase the severity of eye spot.

The nature of the long streaks resulting from *Helminthosporium* infection, although not a matter of apparent immediate commercial or economic importance, was a matter of curiosity to all concerned with the problems of eye-spot control. LARSEN (2) had shown very conclusively, at the time of carrying through the rules of proof connecting this *Helminthosporium* (*Cercospora*) with eye spot of sugar cane, that although the fungus could be isolated and re-isolated from the area within a centimeter or so of the point of infection, it could not be recovered from the long streaks which subsequently elongated from the point of infection.

¹ The identity of *Helminthosporium sacchari* Butler with *Cercospora sacchari* Van Breda de Haan, is a matter solely of assumption by cane pathologists; the question of identity has never been carefully examined although such identity seems probable.

Experimental results

EXPERIMENT I

INTERRUPTION OF FLOW OF NUTRIENTS NOT THE CAUSE OF LEAF STREAKS.—The theory was first advanced that the fungus after gaining entrance to the leaf kills a small area of the host tissue which is in immediate contact with the fungus hyphae, and that this killed area then shuts off the flow of nutrients and water up and down the leaf. From this interference result the streaks which emanate from the original point of infection. To prove or disprove this theory ten leaves of the sugar cane variety Hawaii 109, a very susceptible variety, were selected, and with a sharp sterile scalpel incisions from 0.5–1.5 cm. in length were made transversely across the leaves, thus cutting the vascular systems of the parallel-veined cane leaves and interrupting the flow of nutrients. All such incisions were without effect, and gave no indications of killing or streaks whatsoever. The experiment was repeated with identical results. These results indicated that the streaks resulting from *Helminthosporium* infection were not the result of an interruption of the nutrient supply.

The direction of the investigation then turned rather naturally to the possibility of the production of toxic substances by the eye-spot fungus, *Helminthosporium sacchari*.

EXPERIMENT II

TESTS FOR TOXIC SUBSTANCES IN FILTRATES OF CULTURES IN PURE SUCROSE SOLUTION.—An attempt was then undertaken to determine the presence of a toxic substance in cultures of *H. sacchari*. The first attempts were with cultures of the fungus in solutions of 10 per cent. pure sucrose in water, autoclaved, in 500-cc. Erlenmeyer flasks. Growth in this medium was not vigorous; on the contrary it was rather sparse. At the end of 30 days the cultures were passed through Chamberland filters which separated the fungus from the culture solutions. Flasks of the filtrate were held for 10 days after the filtration and showed their sterility by absence of growth, either of *H. sacchari* or contaminating organisms. If a toxic substance had been produced by the fungus in the cultures it was expected that it would diffuse into the culture solutions, would pass through the filters while the fungus was withheld, and would evidence itself when inoculated into leaves of a susceptible cane variety. However, on inoculation of such filtrate into both young and old leaves of sugar cane, entirely negative results were secured.

In this and subsequent inoculation work both hypodermic needles and capillary tubing were used to introduce the filtrate into the leaves. The capillary tubing when filled with the filtrate was made to puncture the cells of the leaves and allowed to remain thus during the period of the experi-

ment. Unless otherwise noted, all tests for the toxic substance were made upon leaves of the very susceptible sugar-cane variety, H 109, the H standing for Hawaii, this variety being a seedling developed by the cane breeders of the Experiment Station of the Hawaiian Sugar Planters' Association.

Thinking that the toxic substance might not be in sufficient concentration, the filtrate was placed in desiccators over CaCl_2 , and reduced to approximately one-fifth of its original volume, consequently increased to five times its original concentration. Such concentrated portions of the filtrate when inoculated into leaves of H 109 also gave entirely negative results.

EXPERIMENT III

ATTEMPT TO DETERMINE THE PRESENCE OF A TOXIC SUBSTANCE IN FILTRATES OF CULTURES IN NUTRIENT BEEF BOUILLON.—Thinking that the negative results with filtrates of cultures of *H. sacchari* in pure sucrose solutions might be due to the sparse growth of the fungus and sparse formation of a toxic substance, the experiment was repeated with cultures in nutrient beef-extract bouillon. The fungus was grown for 50 days in such cultures, then separated from the culture solutions by the use of Chamberland filters and the filtrates then concentrated to one-tenth their original volume over CaCl_2 in desiccator jars. The absence of *H. sacchari* and contaminating organisms was shown by holding the filtrate in sterile flasks for 10 days.

This concentrated filtrate was then inoculated into leaves of the H 109 cane variety. Then inoculations were made into young actively growing leaves and ten inoculations were made into older, more hardened mature leaves. After thirty days all inoculations but two were definitely negative. The two possibly positive results showed small red streaks, 1–2 mm. in width, one but 5 mm. in length and the other 24 mm. in length. On the basis of these results it could not possibly be concluded that a strong toxin was formed in the nutrient-bouillon cultures. At this stage of the investigation, the possibility that a toxic substance produced by *H. sacchari* was the cause of the leaf stripes of eye spot, appeared rather improbable.

EXPERIMENT IV

TO DETERMINE THE PRESENCE OF A TOXIC SUBSTANCE IN CULTURES IN RICHARD'S SOLUTION.—At this time the writer was re-reading the work by BRANDES (1) on banana wilt and noted the use by BRANDES of Richard's solution; this solution contains 10 gm. KNO_3 , 5 gm. KH_2PO_4 , 2.5 gm. MgSO_4 , 20 mg. FeCl_3 , and 50 gm. sucrose, in 1000 cc. of distilled water. The eye-spot fungus was grown in pure culture in flasks of this solution for 50 days. The cultures were then passed through a Chamberland filter, the culture solutions and diffused products of the fungus passing into the

filtrate and the fungus being separated out. The filtrate was a dark-brown clear liquid. Held in sterile flasks for 10 days there was no indication of growth of the *Helminthosporium* or contaminating organisms.

The project being rather discouraging at this point, no effort was made to concentrate the filtrate. And instead of inoculating the filtrate into the leaves by means of a hypodermic needle, or capillary tubing, 5 leaves of the H 109 variety were placed with their freshly cut ends immersed in the culture filtrate in sterile flasks. Identical H 109 leaves were placed in Richard's solution which had not been cultured with the *Helminthosporium*. This was done on May 31st, 1927, at 11:30 A. M. On the same date at 9:00 P. M. the leaves in the filtrate had begun to wilt and in 24 hours the wilt was clear-cut as compared to the control leaves in the uncultured medium. The leaves in the filtrate also showed long, reddish-brown streaks running longitudinally up the leaf similar in color and appearance to the streaks formed in natural cases of eye spot. The tentative conclusions from this experiment quoted directly from the notebook were:

Tentative conclusions.—(1) One would conclude from these preliminary results that a soluble toxic substance is produced by the eye-spot fungus which diffuses out from the fungus into the culture solution; and this toxic substance can be separated from the fungus and, independently of the fungus, produce on H 109 leaves the streak characteristic of eye spot.

(2) From the failures with cultures in pure sucrose solution and in beef bouillon this toxic substance does not seem to be formed in the absence of inorganic nitrogen.

(3) The toxic substance seems to be buffered in the presence of proteins.

On June 2nd, the same experiment was set up using a new set of flasks of the same kind of filtrate made on May 31st.

EXPERIMENT V

(1) TO CORROBORATE THE RESULTS OBTAINED IN EXPERIMENT IV AND (2) TO DETERMINE THE EFFECT OF THE TOXIC SUBSTANCE ON LEAVES OF THE RESISTANT YELLOW CALEDONIA VARIETY.—A 50-day old culture of the eye-spot fungus in Richard's solution was run through a Chamberland filter on May 30th. The filtrate was kept sterile for subsequent use.

On June 2nd, five small wide-mouthed straight-sided bottles of a diameter of 1.5 inches were filled to a height of 1.5 inches with this filtrate. Young freshly cut leaves of H 109 were placed with their butts immersed in the filtrate, one leaf in each bottle; these leaves were, in order of their age, the youngest leaf of the cane just emerged from the central spindle, the leaf lower than the youngest leaf, the next older leaf, the next, and the fifth leaf from the central spindle. In other words five leaves were taken

in order of their age from a stalk of H 109, and their butts immersed in the filtrate of the eye-spot culture, promptly after being cut.

Five identical bottles were filled to the same height, with Richard's solution, which had been uncultured, that is the eye-spot fungus had not been grown upon it and it had been kept sterile. In these solutions were placed leaves of similar age from a similar stalk of H 109 sugar cane. These bottles served as controls on the eye-spot filtrate.

In order to compare the reaction of the filtrate on leaves of a cane variety resistant to eye spot as compared to leaves of the susceptible H 109, leaves were cut from a fresh stalk of the resistant Yellow Caledonia variety, in consecutive ages as with the H 109, and placed in the same bottles as the H 109 leaves. The experiment was set up at 4:00 P. M., June 2nd.

On June 3rd, at 4:00 P. M., there was no wilting of the leaves in either the filtrate or control bottles, but there was a decided reddening of the vascular bundles of the leaves in the filtrate not visible in the leaves of the controls. This reddening seemed to be more noticeable in the Yellow Caledonia leaves than in the H 109 leaves.

On June 4th, at noon, there was a decided wilting of the H 109 leaves in the filtrate which was not evident in the Yellow Caledonia leaves in the filtrate or in either the Yellow Caledonia or H 109 leaves in the controls. The results at this time were recorded photographically. At the time of taking the photograph the leaves were removed from the filtrate and culture solution controls and placed in water.

On June 5th, at 7:00 A. M., there was wilting of both the Yellow Caledonia and H 109 leaves previously in the filtrate but no wilting of either the Yellow Caledonia or H 109 leaves previously in the culture solution controls.

On June 6th, at 9:00 A. M., the results were the same except that the leaves of both H 109 and Yellow Caledonia in the culture solution controls had begun to wilt.

Tentative conclusions.—(1) It seems evident that there is a toxic substance produced by the eye-spot fungus which if grown in liquid culture media will diffuse out into the culture solution and can then be separated from the fungus by filtration through a Chamberland filter.

(2) Yellow Caledonia leaves reacted to the toxic substance considerably more slowly than the H 109 leaves but eventually did react.

EXPERIMENT VI

THE EFFECT OF HEAT UPON THE TOXIC SUBSTANCE OF THE EYE-SPOT FUNGUS.—The filtrate of the culture of the eye-spot fungus in Richard's solution which was used in the experiment of June 2nd, was used in this test. This was done since it was a difficult procedure to separate the toxic substance

from the fungus and it was thought that the filtrate in the previous experiment could be used again.

This filtrate was therefore sterilized in the autoclave at fifteen pounds pressure for twenty minutes. Such an exposure to heat would almost certainly destroy enzyme action and would probably coagulate most proteins. The presence or absence of a reaction to this autoclaved filtrate would therefore indicate whether the toxic substance was of a protein or non-protein nature.

In order to determine more fully the effect of the toxic substance on H 109 leaves of different ages, the youngest leaf, second, third, sixth and ninth leaves on a single stalk were cut off and their bases immersed in the toxic solution. Leaves in the same order of age were also selected from another stalk and placed with their butts in a non-cultured Richard's solution as controls.

June 8th, this experiment was set up at 11:50 A. M.

June 9th, there was no obvious effect at 7:00 A. M. At 3:00 P. M. youngest leaf in filtrate showed wilting; no wilting in other leaves.

June 10th, 7:00 A. M., all leaves were definitely wilted in filtrate, but all leaves remained normal in controls. Wilted leaves also showed reddening of vascular bundles nearest to the midribs.

Tentative conclusions.—(1) It seemed evident that the toxic substance was not of an enzyme nature nor of a protein nature for the exposure of the toxic substance to the autoclave would have destroyed enzymes and coagulated most proteins.

(2) The results rather indicated the presence of a simple inorganic toxic substance as a by-product of the activities of the eye-spot fungus.

EXPERIMENT VII

TO CORROBORATE THE PREVIOUS EXPERIMENT SHOWING THE NON-PROTEIN NATURE OF THE EYE-SPOT TOXIC SUBSTANCE.—The filtrate of the 50-day old culture of the eye-spot fungus in Richard's solution was autoclaved again for 20 minutes at 15 pounds pressure and then diluted with equal parts of distilled water.

June 17th: The experiment was set up at 6:00 P. M. with leaves on stalk in order of their age; 1st, 2nd, 3rd, 6th and 9th. Leaves of the same age were placed in diluted uncultured medium of identical character as controls.

June 18th, noon: Slight wilting of leaves occurred in the filtrates; little or no discoloration was evident.

June 19th, 9:00 A. M.: The oldest (9th) leaf in the filtrate was almost entirely yellow; 6th leaf yellowed but not as much as 9th; also wilted.

1st, 2nd and 3rd leaves in age were all wilted and rolling at edges, but there was no yellowing as yet.

All controls were normal.

June 20th, 7:30 A. M.: All leaves in the filtrate were badly wilted; leaves in controls entirely normal; the results are shown in fig. 1.

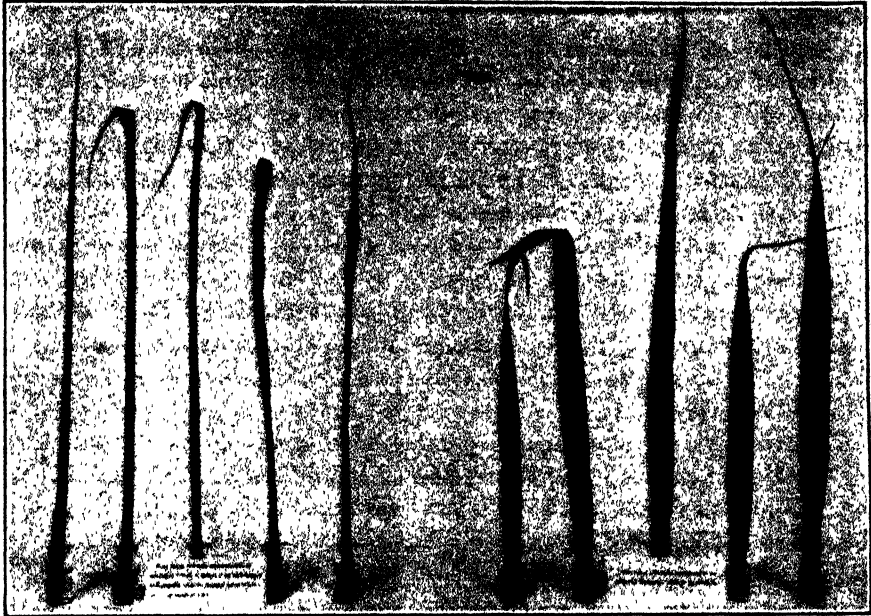


FIG. 1. Effect upon the leaves of the H 109 cane variety of the toxic substance in filtrates of cultures of the eye-spot fungus in Richard's solution. Left: H 109 leaves with butts immersed in twice autoclaved filtrate of culture of eye-spot fungus in Richard's solution. Youngest leaves at left and oldest leaves at right. Right: H 109 leaves with butts immersed in uncultured Richard's solution as controls. The photograph shows the toxic substance still active after autoclaving.

June 23rd, 8:00 A. M.: Experiment concluded.

Conclusion.—The toxic substance of the eye-spot fungus is apparently of a non-protein nature since after being subjected to a high heat its effect upon H 109 leaves is not at all minimized.

EXPERIMENT VIII

TO DETERMINE THE PRESENCE OF NITRITES IN CULTURE FILTRATES.—As a student the present writer had been associated with an investigator (PIERRE BONCQUET) who, in investigating several different plant diseases, showed the presence of considerable concentrations of nitrites within the lesions of

such diseases. The correlation of the presence of nitrites with such other plant diseases also suggested itself as of possible interest in the case of eye spot of sugar cane. Using the tests described in the Manual of the American Society of Bacteriologists,² determinations for the presence of nitrites were therefore made in the filtrates from eye-spot cultures, and as controls, tests were made on the uncultured Richard's solution. The tests were as follows: 5 gm. of sulphanilic acid were dissolved in 150 cc. of acetic acid (sp. gr. 1.04) to make solution A. 1 gm. of α -naphthyl-amine was dissolved in 22 cc. of water, filtered, and 180 cc. of dilute acetic acid added to make solution B. To test for nitrites add 2 cc. each of A and B to 10 cc. of culture to be tested; the presence of nitrites is evidenced by a rose-pink color which should be compared with the absence of such color from controls when tested in an identical manner.

The tests showed considerable concentrations of nitrites in the filtrates of the culture solutions and no nitrites in the controls. Apparently the potassium nitrate of the Richard's solution is reduced by the *Helminthosporium* of sugar-cane eye-spot disease, forming nitrites easily and abundantly. The test was repeated several times and on a number of different cultures.³

The finding of nitrites in considerable concentrations in the culture filtrates and the hitherto rather uncertain association of nitrites with certain plant diseases gave rise to the following experiment.

EXPERIMENT IX

TO TEST THE TOXICITY OF DILUTE SOLUTIONS OF SODIUM NITRITE VERSUS SODIUM NITRATE ON H 109 LEAVES.—The filtrates of cultures of the eye-spot fungus in Richard's solution having been shown to contain a toxic substance of a non-protein nature, and having shown a marked reaction when tested for nitrites, the present test was inaugurated to show if simple nitrites would show the eye-spot reaction as much as the toxic substance from culture filtrates. Sodium nitrate solution of the same concentration as the sodium nitrite solution was used as a check.

June 13th, 4:30 P. M.: Five bottles containing 0.5 per cent. of sodium nitrite in distilled water were used in which to immerse the butts of H 109 leaves. Five bottles of sodium nitrate of the same concentration in distilled

² This manual has not been available to the writer in Manila so that the page reference cannot be given. However, the same test is given in MUIR, ROBERT, and RITCHIE, JAMES. Manual of Bacteriology, p. 356. Oxford University Press, 1921.

³ At this time Mr. ARTHUR F. BELL, of the Bureau of Sugar Experiment Stations of the Queensland Government, was a visitor in the laboratory and appreciation is here expressed to Mr. BELL for the considerable assistance rendered by him in making these tests.

water were run with H 109 leaves as controls. The leaves were placed in these solutions as follows: 1, 2, 3, 6 and 9, in order of their age on the stalk; 1 was the youngest leaf.

June 14th, 7:30 A. M.: The leaves in the sodium nitrite solution were slightly wilted and did not have the luster of leaves in the nitrate solution.

June 14th, 2:00 P. M.: The leaves in nitrite solution showed definite reddening along the midribs.

June 14th, 4:00 P. M.: The wilt was considerably advanced and vascular bundles alongside midribs were definitely reddened in the nitrite solution; leaves in the nitrate solution were entirely normal. The younger leaves were wilted more than older leaves but reddening was equally pronounced in all leaves.

June 15th, 8:00 A. M.: The reaction of the cane in the sodium nitrite was more pronounced; reddening of such leaves was also more pronounced and very definite.

June 16th: Experimental results photographed and shown in fig. 2.



FIG. 2. The effect of the filtrate of the eye-spot fungus in Richard's solution on leaves of H 109 and Yellow Caledonia. In each series there were five leaves in order of their age on the cane stalk; youngest to the left, oldest to the right. The series of five leaves to the left consisted of H 109 leaves in the filtrate; the series to the right were H 109 controls; the third series were Caledonia leaves in the filtrate and the fourth series at the right were Caledonia controls. Photographed four days after being placed in filtrate.

Conclusions.—Solutions of sodium nitrite produced a reaction in H 109 leaves, closely similar to the reaction of the toxic filtrate of cultures of the eye-spot fungus in Richard's solution.

EXPERIMENT X

TO SUBSTANTIATE RESULTS OBTAINED PREVIOUSLY WITH SODIUM NITRITE SOLUTIONS WHICH GAVE A TYPICAL EYE-SPOT REACTION ON H 109 LEAVES, WITH REDUCTION OF CHLOROPHYLL, WHILE LEAVES IN SODIUM NITRATE SOLUTION AS CONTROLS REMAINED NORMAL.—June 23rd, 9:00 A. M.: Three solutions were prepared, the first containing 0.125 per cent. of sodium nitrite in water, the second containing 0.25 per cent. of sodium nitrite in water and the third, 0.25 per cent. of sodium nitrate in water.

Three stalks of H 109 were cut and on each stalk the first, second, third, sixth and ninth leaves in order of their age in the stalk were cut, first being the youngest and ninth the oldest.

Immediately after cutting off these leaves, their butts were immersed in the solutions, the leaves of one stalk in 0.125 per cent. sodium nitrite, the leaves of the second in 0.25 per cent. sodium nitrite and the leaves of the third in 0.25 per cent. sodium nitrate.

June 24th, 8:00 A. M.: The third, sixth and ninth leaves in both concentrations of sodium nitrite showed reddened color along the midribs, in the lower parts of the leaves. There was a slight tendency of the leaves to roll at the edges and wilt.

June 25th, 7:30 A. M.: The older leaves were obviously more severely affected by the sodium nitrite solutions than the younger leaves. The leaves in the 0.25 per cent. solution were more severely affected than the leaves in the 0.125 per cent. solution. The reaction of the leaves was as usual, a reddening of the leaf blades along the midribs, usually more noteworthy in the lower parts of the leaves.

June 26th, 9:30 A. M.: The second, sixth and ninth leaves in the 0.125 per cent. solution of sodium nitrite showed definite yellow streaks typical of the earlier stages of eye spot; these yellow streaks were not found in the more concentrated, 0.25 per cent., solution of sodium nitrite. There were present in both concentrations the red streaks typical of the more advanced stages of eye spot. The control leaves in sodium nitrate solutions were entirely normal.

June 27th: The leaves were now so wilted and rolled as to obscure the yellow and red streaks of the leaves in the sodium nitrite solutions, and the experiment was discontinued.

Conclusions.—Sodium nitrite solutions reproduced the yellow and red streaks in H 109 leaves typical of eye spot. The reaction of sodium nitrite on the leaves was identical with the reaction of the filtrate of cultures of the

eye-spot fungus in Richard's solution. Since Richard's solution contains nitrates which when cultured with the eye-spot fungus produce abundant nitrites, there is a strong inference that the action of the eye-spot fungus on H 109 leaves is to reduce nitrates to nitrites which in turn react on the leaves to form the typical yellow and red streaks which result from eye-spot infection.

The action of the eye-spot fungus and of the sodium nitrite solutions is clearly a reduction of the chlorophyll to form first the yellow color and, with greater concentration, the red color observed in the leaves.

EXPERIMENT XI

TO TEST THE REACTION OF THRICE-AUTOCLOAVED, DILUTED FILTRATE OF THE EYE-SPOT FUNGUS IN RICHARD'S SOLUTION ON H 109 LEAVES.—June 27th: Using the same filtrate of cultures of the eye-spot fungus in Richard's solution, having been autoclaved once more however, making three times in all, an experiment was set up to test the effect of dilution on the filtrate.

One part of the filtrate was diluted with equal parts of water, making a 50 per cent. concentration. The other part of the filtrate was diluted with 3 parts of water, making a 25 per cent. concentration. As a control, uncultured Richard's solution was diluted to a 50 per cent. concentration with water.

June 27th, 4:30 P. M.: Five H 109 leaves were placed in the 25 per cent. concentration of the filtrate; leaves 1, 2, 3, 6 and 8 in order of their age on the stalk. Five leaves from another stalk in order of their age 1, 2, 3, 5 and 8 were placed in a 50 per cent. concentration of the filtrate. Five leaves from another stalk, leaves 1, 2, 3, 5 and 8 in order of their age, were placed in a 50 per cent. concentration of uncultured Richard's solution as controls.

June 28th, 7:00 A. M.: No results were evident as yet.

June 29th, 7:00 A. M.: In the 50 per cent. concentration of filtrate, leaves 3, 5 and 8 were rolled and slightly wilted but not discolored, while leaves 1 and 2 were still normal. In the 25 per cent. concentration of filtrate, leaves 6 and 9 were slightly wilted and edges rolled; leaf 9 was decidedly yellow, especially at the tip; leaf 6 also was yellow at the tip, but less so than 9; other leaves were normal. Control leaves were normal.

June 30th, 7:30 A. M.: All leaves in both 25 and 50 per cent. concentrations of the filtrate were severely wilted; control leaves were normal.

July 1st: The leaves the same as on June 30th; the experiment discontinued.

Conclusions.—The results of this experiment showing that the filtrate of the cultures was still toxic after three exposures to high heat in the autoclave indicated that the toxic substance was of a non-protein nature. The

results also indicated that older leaves of H 109 were more susceptible to the toxic substance than young leaves. Finally, the experimental results also indicated that a more typical eye-spot reaction was obtained with a diluted toxic substance than with the more concentrated form.

EXPERIMENT XII

TO SUBSTANTIATE A PREVIOUS EXPERIMENT SHOWING THAT DILUTE SODIUM NITRITE SOLUTIONS PRODUCE A MORE TYPICAL EYE-SPOT REACTION ON H 109 LEAVES THAN STRONGER CONCENTRATION.—July 1st: Solutions were prepared containing 0.125 and 0.25 per cent. of sodium nitrite in water and 0.25 per cent. of sodium nitrate in water.

5:30 P. M.: From a fresh stalk of H 109, counting the youngest, newest unfolded flat leaf as leaf number 1, were cut off leaves numbers, 2, 3, 6, 7 and 9 and their butts immediately immersed in the solution of 0.25 per cent. sodium nitrate. From a similar stalk of H 109 a series of leaves of identical ages were cut and placed in the solution of 0.25 per cent. sodium nitrite. A similar series of leaves of identical ages was cut and the leaf butts immediately immersed in solutions of 0.125 per cent. of sodium nitrite.

July 2nd, 8:00 A. M.: No reaction in any series.

July 3rd, 8:00 A. M.: In the 0.25 per cent. solution of sodium nitrate there was no reaction. In the 0.25 per cent. solution of sodium nitrite all leaves were slightly rolled and showed red streaks, older leaves were more severely affected than the younger leaves. In the 0.125 per cent. solution of sodium nitrite the same reaction was recorded but the reddening was confined to the leaf blades near the midrib at the bases of the leaves.

July 4th, 6:00 P. M.: Fine examples of typical yellow eye-spot streaks were observed in leaves, 2, 3, 7 and 8 in the 0.125 per cent. sodium nitrite solution and in leaves 3, 6 and 7 of the 0.25 per cent. solution of sodium nitrite. The controls in the sodium nitrate solution showed no reaction.

Conclusions.—Sodium nitrite solutions reproduced the yellow and red streaks in H 109 leaves typical of eye spot. Previous experiments and conclusions were corroborated. The older leaves appeared to be more susceptible to the toxic substance than the younger leaves.

EXPERIMENT XIII

TO COMPARE VARIOUS NITROGEN COMPOUNDS AS SOURCES FOR THE TOXIC SUBSTANCE.—In the earlier stages of this experimental work the possibility had suggested itself that since the toxic substance did not react to any appreciable extent in filtrates of cultures of the eye-spot fungus in beef bouillon, while there was an appreciable reaction in the filtrates of cultures in Richard's solution, possibly there was a buffering action of the proteins of the beef-extract bouillon against the toxic substance. This did not seem so

likely after the finding that the toxic substance was principally an inorganic nitrite; however the following experiment was inaugurated before this latter finding was established; the experiment was planned to test the formation or reaction of the toxic substance in filtrates of cultures with various sources of nitrogen and to test the buffering effect of beef-extract proteins on the toxic substance.

Richard's solution was prepared, omitting the potassium nitrate, and divided into five different lots. To one lot was added one per cent. of potassium nitrate; to the second lot one per cent. of asparagin; to the third lot one per cent. of peptone; to the fourth lot one per cent. of beef extract, and to the fifth lot one per cent. of potassium nitrate plus one per cent. of beef extract. There were therefore four different sources of nitrogen of increasing complexity of structure, and in the fifth lot there was a mixture of protein nitrogen with inorganic nitrogen to determine whether or not such protein would have a buffering action upon the toxic substance. All five classes of media were then sterilized in the Arnold sterilizer on three successive days for 20 minutes of steaming each day.

There were 12 Erlenmeyer flasks of each medium and 6 flasks of each medium were inoculated with the eye-spot fungus, the remaining 6 flasks of each series being kept as controls; this experiment was set up on May 19th, 1927.

On August 20th, 92 days later, all cultures were filtered through Chamberland filters, each series of a distinct medium having a separate clean Chamberland filter; the filtrate was received in sterile flasks.

The filtrates of all series of the cultured media were then tested separately for the presence of nitrites, using the sulphanilic acid, α -naphthylamine test; the results were as shown in table I.

TABLE I

PRESENCE OR ABSENCE OF NITRITES IN FILTRATES OF EYE-SPOT CULTURES, AND UNCULTURED CONTROLS, IN MEDIA WITH VARIOUS SOURCES OF NITROGEN

	POTASSIUM NITRATE	ASPA- RAGIN	PEPTONE	BEEF EXTRACT	POTASSIUM NITRATE PLUS BEEF EXTRACT
Filtrates of cultures	++	Trace	-	-	+
Uncultured media as controls	-	-	-	-	-
Weight of mycelial mass filtered from culture: grams	4.24	5.01	6.19	4.72	5.57

The tests showed a very strong presence of nitrites in the medium with potassium nitrate as a source of nitrogen; a lesser reaction for nitrites in

the medium with mixed potassium nitrate and beef bouillon and but a trace of reaction from the medium containing asparagin. There was no reaction for nitrites in the media containing peptone or beef extract alone.

In this connection it is interesting to note that the mycelial masses from the different series of media were weighed and for convenience such weights are also given in table I. These figures show that there is no correlation between quantity of growth and formation of the toxic nitrites. Rather it is evidently the source of nitrogen which is the factor governing the formation of nitrites. Apparently the fungus of eye spot of sugar cane grows more vigorously on organic forms of nitrogen than on inorganic forms. As against this, however, the fungus seems to have little effect in reducing such more complex forms of nitrogen to nitrites but does have a strong capacity to reduce inorganic nitrates to nitrites; apparently it is this latter factor in its physiology which causes it to be a serious pathogen to certain of the sugar-cane varieties.

The filtrates of the different series of culture media were then diluted with equal parts of sterile distilled water, placed in the straight-sided bottles used in previous experiments, and five leaves of similar degrees of age from identical stalks of the variety H 109, placed with their cut ends or butts in such filtrates. The results of this test are shown in table II.

The results of these toxic substance reaction tests correlate closely with the results of the tests for nitrites in these filtrates and controls, the one exception being that, whereas the qualitative determination for nitrites by the sulphanilic acid test gave no reaction in the filtrates of cultures in peptone medium, there was a slight reaction on the part of the leaves in such filtrates, indicating the presence of this toxic substance; apparently, then, as deduced from previous experiments only very slight quantities of nitrites are necessary to produce a reaction on the leaves of a susceptible cane variety, and one would judge that the leaves give a more delicate test for the presence of nitrites than the sulphanilic acid method.

Conclusions.—The results tabulated in table II would indicate that the eye-spot fungus can produce the toxic substance for H 109 leaves in a medium containing nitrates; this toxic substance is not materially buffered in the presence of the proteins of beef bouillon as was at one time in these studies thought possible; the toxic substance is produced to a lesser extent in a medium containing asparagin, and to an even lesser extent in a medium containing peptone; the toxic substance was not produced in a medium containing beef extract as the sole source of nitrogen.

Review of literature and discussion

The literature concerning the toxic substances formed by plant pathogens is fairly extensive, and no effort will be made to present a complete

TABLE II
REACTION OF LEAVES OF SUGAR CANE, H 109 VARIETY, TO FILTRATES OF 92-DAY-OLD EYE SPOT CULTURES IN VARIOUS MEDIA. EXPERIMENT SET UP AT 9:30 A. M., AUGUST 27TH, 1927

DATE	POTASSIUM NITRATE UN- CULTURED CONTROLS	FILTRATE OF POTASSIUM NITRATE CULTURES	FILTRATE OF ASPA- RAGIN CULTURES	FILTRATE OF PEP- TONE CUL- TURES	FILTRATE OF BEEF EXTRACT CULTURES	FILTRATE OF POTASSIUM NITRATE PLUS BEEF EXTRACT CULTURES
Aug. 28th, 8:00 A. M.	All normal	All normal	All normal	All normal	All normal	All wilted ²
5:00 P. M.	All normal	All wilted	All wilted	All slightly wilted	All normal	All normal
Aug. 29th, 7:00 A. M.	All normal	All wilted ¹	All wilted not yellow	All wilted ²	All normal	All wilted

¹ Leaf 8 shows yellow streaks typical of natural eye-spot lesions; all other leaves are wilted without yellowing.

² Leaves 6 and 8 completely yellowed as in natural eye-spot lesions.

bibliography of the subject, but mention of a few recent papers will suggest the most recent viewpoints.

BRANDES (1) working on the *Fusarium* wilt of banana plants in 1919 expressed what seemed to be the viewpoint from gradually accumulating evidence at that time for several wilt diseases, that "wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus."

WHITE (7) showed the presence of toxic substances in filtrates of cultures of *Fusarium lycopersici*. He apparently arrived at the conclusion that there were two toxic principles in such filtrates; the first, of the nature of an enzyme, and the second crystalloidal and thermostable. He believed that among the organic acids present in the filtrates, might be found the thermostable toxic substance.

ROSEN (5) showed that sterile filtrates of cultures of *Fusarium vasinfectum*, the pathogen of cotton wilt, in Richard's solution (nitrogen in the form of nitrates) were toxic to cotton plants. Filtrates of cultures of the same organism in Uschinsky's solution (nitrogen in the form of asparagin) or peptone bouillon (nitrogen in the form of peptones) were non-toxic to cotton plants. Whole plants with normal uninjured roots when placed in filtrates of cultures in Richard's solution, exhibited the results of the toxic substance, showing absorption of the toxic substance through the roots. These toxic filtrates gave positive tests for nitrites and using chemically pure sodium nitrite solutions of concentrations comparable to the concentrations found in the culture filtrates, ROSEN obtained a reaction by his cotton plants similar to the toxic effect exhibited by his culture filtrates. ROSEN also came to the conclusion that there were two toxic substances formed by the *Fusarium* of cotton wilt: the first was an inorganic salt in the form of a nitrite and the second a volatile compound with an alkaline reaction.

In his paper, ROSEN suggests that cotton wilt which is more prevalent in his experience on sandy soils may be correlated with the lack of organic nitrogen in such soils, basing this deduction on the presence of the toxic substance in cultures containing inorganic nitrogen and the absence of the toxic substance in cultures containing only organic nitrogen. He goes somewhat further along this line of thought in a later note (6) in which he suggests the possibility of the control of cotton wilt by the use of organic nitrogen fertilizers. The work of BRANDES, WHITE and ROSEN, all showed that the wilting produced in plants placed in culture filtrates as compared to normal plants in controls, could not have been brought about by a change in osmotic pressure of the culture solution which might have resulted from the fungus metabolism.

The results of studies outlined in the present paper are in close agreement with the main body of the conclusions by WHITE and by ROSEN

although there is evidence of the presence of but one toxic substance from the studies here recorded. The toxic substance produced by the fungus of eye-spot of sugar cane has been shown to be a soluble, inorganic substance not affected by heat, and subsequently by identical reactions the inference is strong that this substance is simply an inorganic nitrite. These nitrites are formed readily in media containing inorganic nitrates and to a less extent from lower organic nitrogen forms, such as asparagin, but are not formed at all from peptone, or the higher protein forms of nitrogen. The toxic action of the nitrites is not buffered by the presence of proteins such as those in beef extract.

Apparently the toxic substance is not truly a metabolism product of the pathogen but results solely from the vigorous capacity of the pathogen to reduce to nitrites the inorganic nitrates present in the medium or other environment.

These results obtained in culture and toxin studies fit in admirably with the results by LEE and MARTIN (3) which showed big increases in the virulence of the disease following applications of inorganic-nitrogen fertilizers to fields of susceptible cane varieties, even though the nitrogen is applied in the form of ammonium sulphate rather than sodium or potassium nitrates. Apparently the nitrogen taken up by the cane plant arrives in the cane foliage in the form of nitrates, which are reacted upon vigorously by the eye-spot fungus to produce nitrites abundantly, which are in turn toxic to the cane-leaf tissues. One would judge that one important effect of the nitrates on the leaf tissues was the reduction of the chlorophyll as indicated by the color changes from green to yellow and red in streaks on the leaves. It is of possible physiological interest that this reduction takes place more strongly in the older leaves than in the younger ones.

As to the application of nitrogen in organic forms to minimize the disease or more properly to avoid the formation of toxic substances as suggested by ROSEN, the question would reasonably seem to be, whether such nitrogen arrives in the cane leaves in the form of organic compounds or as inorganic nitrates. The field experience with ammonium sulphate and with organic forms of nitrogen fertilizers, such as filter-press mud from sugar-cane factories, or dried blood, etc., has shown the big increases of eye-spot similar to the increases of the disease when inorganic nitrate fertilizers are used, and would seem in opposition to the theory that organic fertilizers minimize the disease.

There is, however, a field application of these experimental results, which has already proven notably successful; when the application of nitrogen fertilizers is made at such a time as to bring the physiological response in the host plant, at a time of the season when climatic conditions for fungus

infection are at a minimum there is scarcely any injury from the disease at all. This has been proven out as a commercially feasible practice in the case of eye-spot of sugar cane.

The tests with the toxic filtrates on leaves of the Yellow Caledonia variety, a variety which is resistant to eye-spot, also led to some interesting inferences. Yellow Caledonia leaves frequently become infected under natural field conditions, but never develop the long streaks of killed tissue found on the susceptible varieties following infection; instead, there are formed only small local lesions at the point of infection. However, when Yellow Caledonia leaves are placed in solutions of the toxic substance, they indubitably react to such a toxic substance. One would conclude therefore that studies of the nitrogen metabolism of a variety such as Yellow Caledonia would possibly show the nitrogen in the leaves, in forms other than the simple inorganic nitrates, so that the fungus had difficulty in producing the toxic nitrites.

At the time of the conclusion of the writer's work on eye-spot, attention was also called to the possibility of fertilizing with catalytic oxidizing agents in an effort to get such catalysts into the nutrient streams of the plant in order to neutralize the reducing effect upon nitrates produced by the pathogen. The salts of such metals as iron, manganese and zinc were suggested for such attempts.

The studies at least lead to a more intelligent attempt at producing in the host plant resistance to the toxic effects produced by a certain group of plant pathogens.

Summary

1. Tests for a toxic reaction of susceptible sugar-cane leaves were conclusively negative when tested with filtrates of cultures of the fungus of eye-spot of sugar cane, *Helminthosporium sacchari*, in pure sucrose solutions and in beef-extract bouillon media.

2. Tests on sugar-cane leaves with filtrates of cultures of the same fungus in Richard's solution showed the presence of a toxic substance. This toxic substance was shown to persist after heating to an extent which would destroy enzymes and coagulate most proteins. Attention rather naturally therefore turned towards simpler nitrogen compounds as the toxic substances.

3. The presence of considerable concentrations of nitrites was shown in the filtrates of cultures of the fungus in Richard's solution and to a lesser extent in an asparagin medium, but such nitrites were not evidenced in filtrates of cultures in a peptone bouillon medium nor in a beef-extract bouillon, nor in uncultured Richard's solution as a control on the test for nitrites.

4. Susceptible sugar-cane leaves evidenced a toxic reaction from filtrates of the cultures in Richard's solution, to a lesser extent in an asparagin medium and to a much slighter extent in a peptone medium. There was no toxic reaction by such leaves to filtrates of cultures in beef-extract bouillon. There was no correlation between quantity of growth in these media, and quantity of the toxic substance produced. The reaction of cane leaves to these filtrates of cultures in media with various nitrogen sources was closely correlated with the presence of nitrites in such filtrates.

5. Dilute solutions of potassium nitrite brought about the same reaction of susceptible sugar-cane leaves as the filtrates of cultures of the eye-spot fungus in media containing the lower nitrogen compounds. This reaction, in dilute concentrations, was a yellowing of leaves either totally or in streaks, and sometimes the formation of brownish-red streaks up and down the leaves. Leaves in these filtrates always wilted and showed a loss of the glossy appearance typical of the control leaves which remained unwilted and a normal green color.

6. The evidence points to this toxic substance, therefore, not as a direct product of the metabolism of the fungus but as a product of the environmental medium. Apparently the fungus of eye-spot of sugar cane has a strong capacity to reduce to nitrites some of the lower nitrogen compounds in its environmental medium; these nitrites in turn are toxic to the leaf tissues of the host plant. One notable toxic effect produced by such nitrites is the reduction of the chlorophyll of the host tissues.

7. The results recorded in this paper indicate a strong relationship between the virulence of this disease, and probably many other plant diseases, to the nitrogen nutrition of the host plant. The attempt to apply these results to commercial crop production by using organic nitrogen fertilizers for the nutrition of the host plants depends for its success on the nitrogen from such fertilizers reaching the leaves in a nitrogen form other than as nitrates. Field results with organic nitrogen fertilizers in the case of eye-spot disease of sugar cane indicate as large an increase of the disease as that which follows applications of inorganic nitrogen fertilizers. The logic of the results recorded in this paper as well as of the field results does not therefore indicate that applications of organic nitrogen fertilizers will serve as a control measure for this disease.

8. A practical application has been developed for minimizing this disease which in principle is dependent on avoiding nitrogen fertilizers during seasonal periods in which climatic conditions favor fungus infection; quite the reverse, nitrogen fertilizers of any class should be applied at a time when the host plant will react to the nitrogen in a period with climatic

conditions unfavorable to infection. This procedure has been notably successful in actual practice against eye-spot disease of sugar cane.

PHILIPPINE SUGAR ASSOCIATION,
MANILA, PHILIPPINE ISLANDS.

LITERATURE CITED

1. BRANDES, E. W. Banana wilt. *Phytopath.* **9**: 339-389. 1919.
2. LARSEN, L. D. The eye-spot disease. *Hawaiian Planters' Record* **7**: 18-30. 1912.
3. LEE, H. ATHERTON, and MARTIN, J. P. Effect of fertilizer constituents on the eye-spot disease of sugar cane. *Jour. Ind. Eng. Chem.* **20**: 220-224. 1928.
4. MARTIN, J. P., and LEE, H. ATHERTON. The time of application of nitrogen fertilizers in relation to eye-spot disease. *Hawaiian Planters' Record*. **32**: 9-11. 1928.
5. ROSEN, H. R. Efforts to determine the means by which the cotton-wilt fungus, *Fusarium vasinfectum*, induces wilting. *Jour. Agr. Res.* **33**: 1143-1147. 1926.
6. ———. The control of cotton wilt by the use of organic fertilizers. *Science n. s.* **65**: 616-617. 1922.
7. WHITE, RICHARD P. Studies on tomato wilt caused by *Fusarium lycopersici* Sacc. *Jour. Agr. Res.* **34**: 197-239. 1927.

THE ABSORPTION OF PHOSPHATE FROM SOIL AND SOLUTION CULTURES¹

L. J. H. TEAKLE

(WITH FOUR FIGURES)

Introductory

In the study of the phosphate nutrition of plants it is of importance to know what relation exists between the soil solution and the absorption of phosphate by the growing plant, concerning the power to do which, there is a difference of opinion. Some emphasize the effect of carbon dioxide arising from the respiration of the roots; others suggest an internal factor related to the metabolism of the plant. The question of the rate of supply as well as the effect of concentration is of great importance in the interpretation of results of soil and solution culture experimentation. This has been overlooked by certain of the earlier plant physiologists and has led to an emphasis of the importance of the action of the roots on the soil particles. There is, however, ample experimental evidence to show the ability of plants to absorb phosphate from solutions comparable in phosphate concentration with the soil solution. Thus BIRNER and LUCANUS (4) and BEYER (3) as early as 1870 had shown the ability of oat plants to make normal growth in well water containing 2 ppm. PO_4 and SCHLOESING fils (13) and KOSSOWITCH (9) arrived at similar conclusions some thirty years later. SCHLOESING fils found 1 ppm. PO_4 adequate for certain plants and concluded that the soil solution supplied at least the major part of the phosphate absorbed from the soil. KOSSOWITCH recognized a difference between flax and mustard and suggested that this was due to effect of the carbon dioxide formed by the roots. HOAGLAND and MARTIN (7), using solution cultures, obtained good growth of barley at 0.7 ppm. PO_4 . Somewhat better growth occurred in similar solutions containing 2.4 ppm. and 9.0 ppm. PO_4 .

The rôle of calcium carbonate in limiting absorption from rock phosphates has been indicated by PRIANISCHNIKOW (12), BREAZEALE (5) and others. Apparently the carbonic acid arising from root respiration is neutralized by the calcium carbonate with the formation of calcium bicarbonate which depresses the solubility of calcium phosphate. It seems that carbonic acid *may* be an important agent in the solution of soil phosphates, but

¹ Contribution from the Laboratory of Plant Nutrition, the University of California. This problem was suggested by Professor JOHN S. BURD, to whom grateful acknowledgment is made for helpful counsel throughout the investigation.

in highly buffered, neutral or slightly alkaline soils the plant must draw its supply from the phosphate of the soil solution. A recognition of this difference may lead to an explanation of the different results obtained under varied circumstances and to a reconciliation of various theories.

Experimental

This series of experiments was designed to afford evidence concerning the relation between the phosphate concentration of the soil solution and the absorption of that ion by the wheat plant.

For this purpose, Pusa 4 wheat, obtained from the University Farm, Davis, California, was used on account of its early maturity. It was grown in soil cultures and in solution cultures, and, after harvesting, the plants were weighed and absorption determined by analysis. All experiments here reported were conducted during the summer of 1926 in the same greenhouse at the University of California, Berkeley, California. The methods in common use in the laboratories of Plant Nutrition, University of California, were employed throughout. Brief mention may be made as follows:

The soil solution was obtained by the water displacement method of BURD and MARTIN (6).

Phosphate, when in sufficient concentration, was determined by titration of the phospho-molybdate precipitate. For small concentrations, and for the control of the solution cultures, the coeruleo-molybdate method described by ATKINS (2) was used.

To obtain the ash for analysis, samples of the dried and ground plant materials were weighed, mixed intimately with magnesium oxide, and ignited to a dull red heat in an electric muffle.

A. SOIL CULTURES

Three soils were selected for the cultural experiments on account of their physical condition and the phosphate concentration of the soil solution. They were all amenable to the displacement method. Certain details of their history and composition are given in table I.

It should be added that each soil was neutral or nearly neutral in reaction and exhibited a strong buffer action toward acid. Further details of this behavior are reported in another paper (15).

The soils were subjected to various treatments in order to modify the concentration of the soil solution with respect to phosphate. In the case of soils 29 and 30, the calculated amounts of hydrochloric acid and chlorides of calcium and magnesium were added to the distilled water used in moistening the dry soil.

On account of the poverty of soil 33 (see table II) a culture solution, 0.01 molal with respect to potassium, calcium, magnesium, and sulphate

TABLE I
DESCRIPTION OF SOILS USED THROUGHOUT THE EXPERIMENTATION

LABORATORY SERIAL	SOIL SERIES (CALIF. SURVEY)	ORIGIN	PAST TREATMENT	TOTAL PO_4 (DRY BASIS) <i>Per cent.</i>
29	Oakdale sandy loam	Stanislaus Co. J. Skitstone Ranch, Salida, California	Uncropped edge of a vineyard	0.24
30	Fresno fine sandy loam	San Joaquin Valley, G. Christensen Ranch, Fresno, California	Originally grain Edge of vineyard during last 24 years	0.18
33	Not mapped, sandy loam	Creek bottom, University Farm, Davis, California	None	0.16
33a	Not mapped, sandy loam	Creek bottom, University Farm, Davis, California	Intimately mixed with 0.085 per cent. PO_4 as ground rock phosphate	0.24

TABLE II
SOIL 33. ANALYSIS OF THE DISPLACED SOLUTION

MOISTURE	SPECIFIC RESISTANCE	pH	PO ₄	NO ₃	Ca
<i>Per cent.</i>	<i>ohms</i>		<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>
19	2250	7.8	0.2	52	50

and 0.03 molal with respect to nitrate, was used instead of distilled water to moisten the soil.

After standing for about one week, the cultures were seeded to obtain a stand of six healthy plants per culture. Three of these plants were harvested at the intermediate period. For each treatment, five two-gallon, glazed earthenware crocks, each containing 10 kilograms of soil, were used. The moisture was maintained by additions of distilled water in quantities determined by the loss in weight of the jars. Uncropped controls were used in connection with all experiments.

At the beginning of the experiment and at the two other periods during the growth of the plants, the soil solution was obtained from samples of soil under each treatment and from the controls. The results are shown in the accompanying tables and are considered to afford a fairly accurate picture of the fluctuations of the phosphate concentration of the soil solution under the several treatments.

The treatments of the soils and certain of the effects of these treatments on the composition of the soil solution are given in table III. All determinations were made in duplicate. The amendments were calculated on the basis of the theoretical soil solution and are expressed in terms of normality, or as parts per million of the soil solution. Another paper (TEAKLE 15) deals more exhaustively with the chemical effects of these amendments.

In table IV are presented the data concerning the growth and analysis of the wheat plants under the several conditions. All ash analyses reported are the averages of closely agreeing triplicate determinations, and the weights given are averages of a harvest of at least 15 plants from each treatment.

Apparently healthy plants were produced, but there was a marked difference in tillering under the different treatments, which is reflected by the weights. No doubt the high salt content of the soil solution, induced by certain of the treatments, was a seriously complicating factor, and it will be impossible to use any of the data except those obtained under favorable conditions. It seems permissible to consider the effects of the treatments of soil 33 (including 33a), and also the results from soils 29 and 30 involving the milder treatments. Too little is known of the effect

TABLE III

SOILS 29, 30, 33. THE EFFECT OF AMENDMENTS ON THE COMPOSITION OF THE SOIL SOLUTION DISPLACED FROM SOILS WITH AND WITHOUT A CROP OF WHEAT, 1926

SOIL	CHEMICAL TREATMENT	CROP	PLANTING				FIRST HARVEST				FINAL HARVEST			
			DATE	pH	PO ₄	NO ₃	DATE	pH	PO ₄	NO ₃	DATE	pH	PO ₄	NO ₃
29	Distilled water	- +	May 5	6.6	ppm. 7.3	ppm. 550	May 29	6.8 7.2	ppm. 2.4 2.6	ppm. 600 21	June 11	7.3 7.8	ppm. 2.3 4.4	ppm. 635 12
29	0.08 N.HCl	- +	May 5	6.2	12.4	218	May 29	6.1 6.6	8.6 6.6	265 48	June 11	6.9 6.6	5.0 2.4	525 73
30	Distilled water	- +	May 3	7.5	6.5	563	May 29	7.2 7.8	4.3 4.5	810 55	June 11	7.4 8.3	6.4 3.8	728 17
30	0.03 N.HCl	- +	May 3	6.8	12.8	551	May 29	6.8 7.3	9.6 4.0	785 44	June 11	7.2 7.5	11.0 3.0	680 20
30	0.1 N.HCl	- +	May 3	6.0	41.0	471	May 29	5.9 6.4	31.0 19.0	560 350	June 11	6.4 6.8	23.0 13.0	556 180
30	1000 ppm. Ca as Ca Cl ₂ ..	- +	May 8	7.1	3.6	362	May 29	7.2 7.3	3.0 0.8	790 N.D.	June 16	7.5 7.3	4.1 1.6	888 22
30	500 ppm. Mg as Mg Cl ₂ ...	- +	May 8	7.2	5.2	298	May 29	7.2 7.0	3.4 0.9	686 N.D.	June 16	7.0 7.3	3.4 1.7	752 26
33	Culture solution minus PO ₄	- +	June 30	7.5	0.16	1760	August 12	7.4 7.6	0.19 0.15	1750 276
33a	Culture solution minus PO ₄	- +	June 30	7.5	0.15	1870	August 12	7.5 7.8	0.17 0.21	1590 126
33a	Culture solution plus 1390 ppm. PO ₄	- +	June 30	7.2	23.0	1800	August 12	7.4	11.1	1540
												7.8	12.5	108

TABLE IV

SOILS 29, 30, AND 33. GROWTH AND COMPOSITION OF WHEAT PLANTS IN THE SOIL UNDER THE TREATMENTS DESCRIBED IN TABLE III. VARIETY PUSA 4

SOIL	TREATMENT	DATE PLANTED	FIRST HARVEST					FINAL HARVEST						
			DATE	AGE	SOIL SOLUTION PO_4	PLANTS			DATE	AGE	SOIL SOLUTION PO_4	PLANTS		
						DEY WEIGHT	PO_4	PO_4 ABSORBED PER PLANT				DEY WEIGHT	PO_4	PO_4 ABSORBED PER PLANT
				days	ppm.	gm.	per cent.	mg.		days	ppm.	gm.	per cent.	mg.
29	Distilled water	May 5	May 29	24	2.6	0.36	3.10	1.12	June 11	37	4.4	1.01	2.76	2.79
29	0.08 N.HCl	May 5	May 29	24	6.6	0.14	4.45	0.62	June 11	37	2.4	0.87	3.40	2.96
30	Distilled water	May 3	May 29	26	4.5	0.48	2.75	1.32	June 11	39	3.8	1.84	1.96	3.61
30	0.03 N.HCl	May 3	May 29	26	4.0	0.48	2.75	1.32	June 11	39	3.0	2.07	1.92	3.97
30	0.1 N.HCl	May 3	May 29	26	19.0	0.22	4.40	0.97	June 11	39	13.0	1.03	3.27	3.37
30	1000 ppm. Ca as CaCl_2	May 8	May 29	21	0.8	0.24	2.54	0.61	June 16	39	1.6	1.88	1.64	3.08
30	500 ppm. Mg as MgCl_2	May 8	May 29	21	0.9	0.33	2.34	0.77	June 16	39	1.7	2.33	1.58	3.68
33	Culture solution minus PO_4	June 30							August 12	43	0.15	0.79	1.18	0.93
33a	Culture solution minus PO_4	June 30							August 12	43	0.21	0.90	1.18	1.06
33a	Culture solution plus 1390 ppm. PO_4	June 30							August 12	43	12.5	1.27	2.26	3.08

of balance of ions on absorption to permit of conclusions from results obtained under more severe conditions.

In considering soil 33 it seems apparent that the addition of a considerable amount of finely ground rock phosphate, which affected the concentration of the soil solution but little, had a correspondingly small effect on the absorption of phosphate by the plants. In soils of this nature the effect of carbon dioxide on the solid phase phosphate must be very small. In addition there appears to be no definite relation between the concentration of the soil solution and the amount of phosphate absorbed therefrom. The effect of concentration in this regard will be studied under more favorable conditions in solution culture, using concentrations suggested by a study of these and of other soils.

The treatment of soil 30 with CaCl_2 and MgCl_2 suggested that calcium in the soil solution retards the rate of solution of solid phase phosphate to replace that absorbed by plants. While the solubility of phosphate is lowered materially by the presence of increased amounts of calcium in the soil solution, according to the data in table V, it seems that this retardation is the more important effect in soil 30. It will be noted that on account of the phenomenon of base replacement the application of magnesium chloride gave practically the same results as an equivalent amount of calcium chloride.

B. SOLUTION CULTURES

It seemed desirable to conduct accurate controls in the shape of solution cultures. A constant flow device was therefore arranged as shown in fig. 1. Solution of carefully maintained composition was allowed to flow from the upper carboys (A) into the culture jars (B), being delivered at the bottom. The overflow was removed by means of a J siphon after the design of ANDERSEN (1) and caught in carboys (C) similar to the first. After analysis of the solutions in C, they were adjusted to the original concentrations and pumped into the upper carboys (A) by means of a small Nelson pump. The volume was adjusted with distilled water to 40 liters in these upper carboys. The rate of flow was controlled by glass stop cocks (D) and varied from 1 liter to 8 liters per day per plant depending on the composition of the solution and the age of the plants. It was found necessary to coat the containers and conducting tubes with a black asphaltum paint to exclude light. This necessitated a sheath of pasteboard or several thicknesses of brown paper to maintain the solutions at an even temperature. The culture jars (B) had a capacity of about 16 liters of solution and supported a growth of 10 or 20 wheat plants; the number being determined by the concentration of phosphate in the solution.

The carboys (A and C) each had a capacity of 40 liters and were graduated in units of two liters to facilitate control of the rate of flow. All

TABLE V
SOIL 30. THE EFFECT OF A HIGH CONCENTRATION OF CALCIUM IN RETARDING THE SOLUTION OF SOIL PHOSPHATE DURING THE GROWTH OF WHEAT PLANTS
SOILS MOISTENED MAY 6TH

SOIL TREATMENT	ANALYSIS OF THE DISPLACED SOLUTION											
	MAY 8TH			MAY 29TH			JUNE 16TH, 1926					
	PO ₄		Mg	PO ₄		UNCROPPED	PO ₄		Ca		Mg	
				CROP	UNCROPPED		CROP	UNCROPPED	CROP	UNCROPPED	CROP	UNCROPPED
1 Control	ppm. 6.5	ppm. 188	ppm. N.D.	ppm. 4.5	ppm. 4.3	ppm.	ppm. 3.8	ppm. 6.4	ppm. 84	ppm. 240	ppm. 30	ppm. 67
2 1000 ppm. Ca as CaCl ₂	3.6	1020	210	0.8	3.0		1.6	4.1	707	1080	141	209
3 500 ppm. Mg as MgCl ₂	5.2	576	270	0.9	3.4		1.7	3.4	450	660	229	284

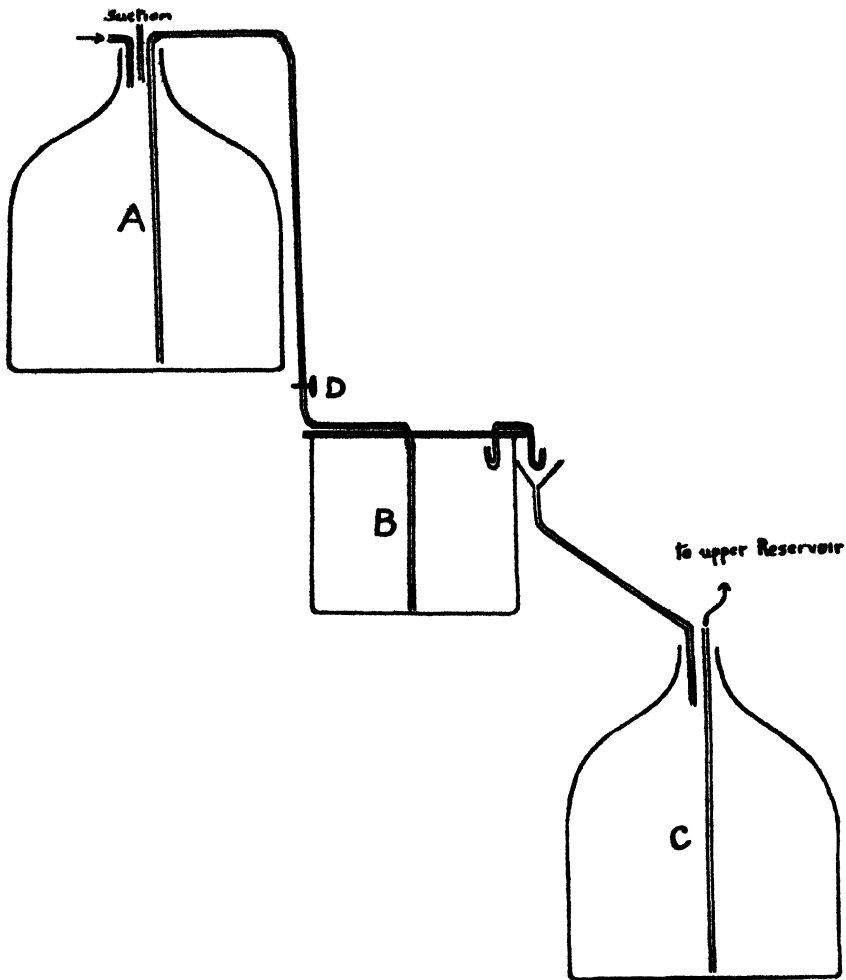


FIG. 1. Diagrammatic representation of a device for the constant renewal of culture solution used in the study of the growth of wheat.

experiments described were conducted side by side with the soil cultures described in the foregoing section.

Harvesting took place on the dates reported in table VI. The date of the final harvest was determined by the emergence of the anthers from the glumes; that is, the plants were harvested at the beginning of the flowering stage.

In all essentials the plants were treated in the same way as those grown in soil cultures.

Three experiments were conducted using this method and were designed to determine the growth and composition of wheat plants in solutions containing phosphate ranging from 0.05 ppm. PO_4 to 50 ppm. PO_4 , the reaction being maintained at either pH 5.5 or pH 7.0. The results of these experiments are reported in table VI. The results of harvesting at an intermediate stage are not included in this table as not contributing to the discussion.

In all cases the culture solution at the beginning of the experiments was 0.01 molal with respect to potassium, calcium, magnesium, and sulphate, and 0.03 molal with respect to nitrate. This concentration was not unduly changed in consequence of the growth of the plants. Phosphate was maintained by use of amounts of carefully standardized KH_2PO_4 indicated by analysis. The pH was maintained by use daily of normal solutions of potassium hydroxide and sulphuric acid. Iron was added daily, using 2 cc. of 0.5 per cent. ferric tartrate per culture. One part per million of manganese and boron were added to the solutions at the beginning of each experiment.

Fine healthy plants were produced under all of these conditions. In the presence of 50 ppm. PO_4 at pH 7.0 slight chlorosis was noted, but apparently no untoward results occurred. At least one good head was produced per plant in each treatment and it was significant that, while about three head-bearing stalks were produced at 1 ppm. PO_4 or above, practically all plants were limited to one head-bearing tiller at lower concentrations. Also, at these lower concentrations the roots were long and spindling and were discolored, suggesting a deposit of iron oxide. In other cultures the roots were strong and lustrous.

THE EXPRESSED SAP

Having sufficient green material available it was decided to make a study of the expressed sap of the wheat plants grown under these carefully controlled conditions. After taking the green weights, half of the plants were systematically selected and cut in suitable lengths for handling. The material was bottled, "killed" by freezing at -15°C ., and the expressed sap obtained under a mechanical pressure of 200 kilograms per square centimeter immediately after thawing.² This method has been used successfully by many investigators among whom may be mentioned NEWTON (10) and HOAGLAND and MARTIN (8) of this laboratory.

Immediately after the pressure was released the sap was filtered and titrated electrometrically against 0.1 N $\cdot \text{H}_2\text{SO}_4$ and 0.1 N $\cdot \text{NaOH}$, using the hydrogen electrode and converting the E.M.F. readings to pH by use of

² The author is indebted to the Division of Pomology, University of California, for the use of the cold chambers and press required for this operation.

TABLE VI
EXPERIMENTS 1, 2, AND 3
DETAILS CONCERNING THE GROWTH AND COMPOSITION OF WHEAT PLANTS GROWN IN FLOWING CULTURE SOLUTION

CULTURE NO.	EXPERIMENT 1				EXPERIMENT 2			EXPERIMENT 3			
	1 (CON- TROL)	2	3	4	5	6	7 (SUB- CON- TROL)	8	9	10	11 (SUB- CON- TROL)
Culture solution, pH...	5.5	7.0	5.5	7.0	5.5	5.5	5.5	5.5	7.0	5.5	5.5
PO ₄ ppm.	5.0	5.0	50.0	50.0	0.05	0.5	5.0	0.1	0.1	1.0	5.0
Date of germination	June 7	June 7	June 7	June 7	July 17	July 17	July 17	Sept. 1	Sept. 1	Sept. 1	Sept. 1
Date of planting	June 14	June 14	June 14	June 14	July 24	July 24	July 24	Sept. 7	Sept. 8	Sept. 8	Sept. 8
Date of harvest	July 23	July 23	July 23	July 23	Sept. 8	Sept. 8	Sept. 8	Oct. 16	Oct. 18	Oct. 18	Oct. 21
Age (days)	39.	39.	39.	39.	46.	46.	46.	38.	40.	40.	43.
Tops, dry weight per plant (gm.)	2.08	1.56	1.95	2.11	0.74	0.76	1.86	0.87	0.83	1.93	2.36
Moisture, per cent.	83.7	84.7	84.0	83.3	82.9	86.2	83.9	79.4	80.8	83.5	83.2
PO ₄ , per cent.	2.26	2.17	2.26	2.17	1.19	2.90	2.49	0.68	0.87	2.40	2.53
PO ₄ , absorbed per plant (mg.)	47.0	33.8	44.1	45.7	8.8	22.0	46.3	5.92	7.22	46.3	59.6
Roots, dry weight per plant (gm.)	0.29	0.25	0.28	0.26	0.16	0.10	0.29	0.26	0.23	0.29	0.34
PO ₄ , per cent.	2.35	3.18	3.88	3.88	1.15	2.62	3.60	0.70	0.73	2.52	3.32
PO ₄ , absorbed per plant (mg.)	6.81	7.95	10.9	10.1	1.84	2.62	10.4	1.82	1.68	7.30	11.3
Relative absorption of phosphate											
In entire plant (c.f. 1)	100.	77.8	102.	104.	19.7	45.7	105.	14.3	16.5	100.	132.
In tops only	100.	72.	94.	97.	18.7	47.	98.5	12.6	15.4	98.5	127.
In roots only	100.	117.	160.	148.	27.	38.5	154.	26.8	24.7	107.	166.
No. of tillers per plant.	5.0	3.9	3.7	4.4	2.2	2.6	3.8	1.4	1.9	4.2	4.2
No. head-bearing stalks per plant.	3.7	3.1	3.5	?	1.0	1.0	3.0	1.2	1.1	2.9	2.7

the tables compiled by SCHMIDT and HOAGLAND (14). Ten cubic centimeters of the sap, diluted with 10 cc. CO_2 -free distilled water, served for each titration. Other portions of the sap were analyzed for phosphate (volumetrically and colorimetrically), and also for other ions where possible. The results of the titrations are expressed as curves in figs. 2, 3, and 4, and the analytical data are reported in table VII.

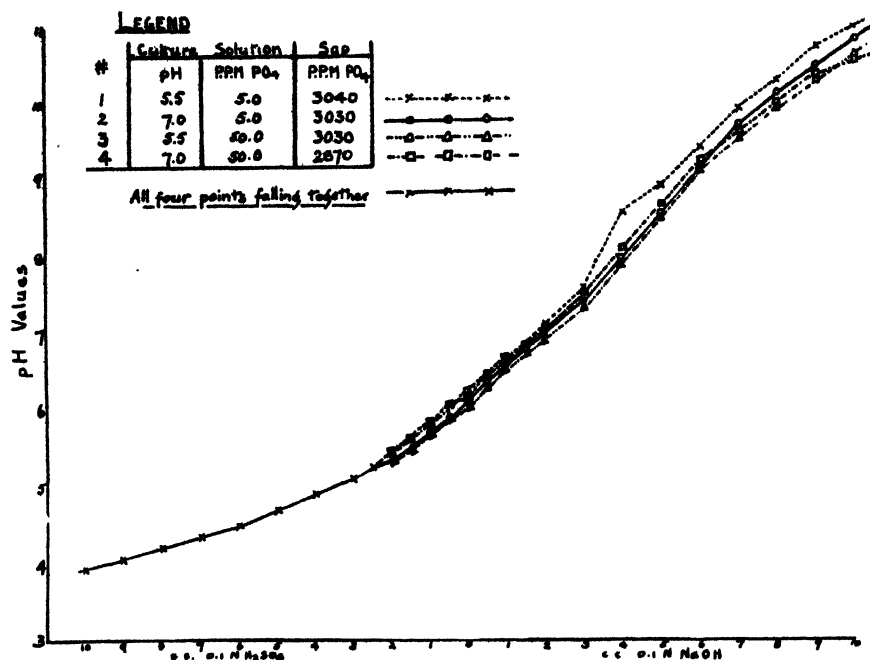


FIG. 2. Titration curves of expressed sap from plants grown in cultures 1, 2, 3, and 4, experiment 1. Between pH 5.3 and pH 3.9 the points so nearly coincided that it was necessary to use a single curve to represent the results, and avoid a confusion of lines.

The results are instructive in showing that the reaction of the sap obtained by these methods is not materially altered by phosphatic treatment or reaction of the culture solution in the range used. The buffer action of the sap, as determined by electrometric titration of the usual type, is markedly influenced, however, on the alkaline side but not on the acid side. This difference may be correlated with the amount of phosphate in the cell sap.

The phosphate of this sap is related to the phosphate of the culture solution, but there seems to be no direct proportionality. The results indicate that the phosphate of the sap is approximately constant when the medium contains between 0.5 ppm. PO_4 and 50 ppm. PO_4 . When the culture solution is more dilute with respect to phosphate the sap seems relatively

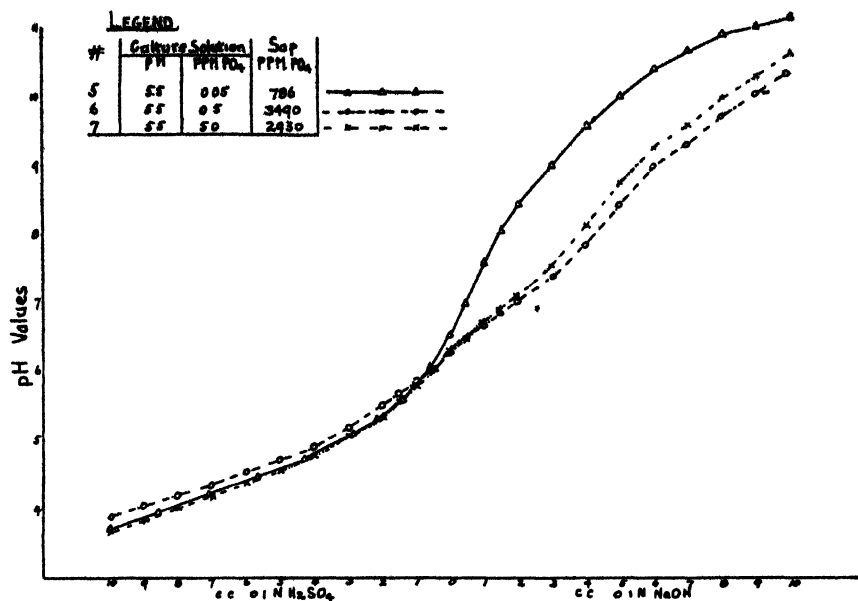


FIG. 3. Titration curves of expressed sap from plants grown in cultures 5, 6, and 7, experiment 2.

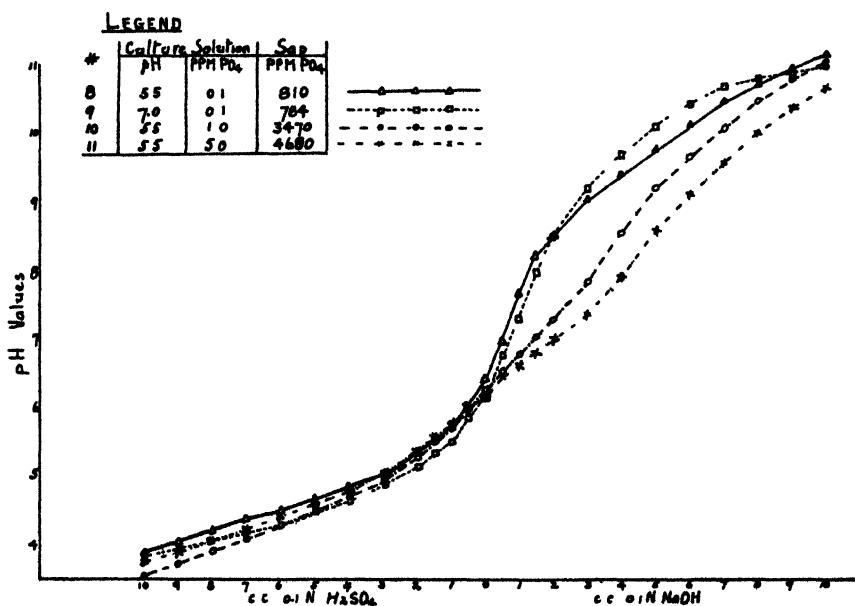


FIG. 4. Titration curves of expressed sap from plants grown in cultures 8, 9, 10, and 11, experiment 3.

constant at about 800 ppm. PO_4 . The non-sap phosphate of the plant does not seem to be related to the concentration of the culture solution. Again, the close approximation of volumetric and colorimetric methods in practically all cases *suggests* that the sap phosphate is inorganic in nature, or is very loosely combined.

A relatively complete analysis of the sap from cultures 10 and 11, experiment 3, enables a calculation of the reaction values. These results indicate a considerable excess of base, suggesting that an organic anion must play an important part in the sap of the plant. What part this anion may contribute to the buffer system of the plant is a matter of conjecture at present, but it seems probable that phosphate plays an important rôle in counter-acting any change on the alkaline side.

Discussion of results

It is unfortunate that the results reported in this paper are too meager in quantity to allow the formulation of definite conclusions or generalizations. Much further study, involving the use of many different varieties of wheat and different types of plants, and a more extensive exploration of the effects of lower concentrations of phosphate, will be necessary before these ends are attained. The method of attack seems worthy of mention to investigators in plant physiology and it is hoped that the suggestions obtainable from the results may assist in future researches.

As yet no one has been able to solve the problem of the phosphate nutrition of plants. That phosphate is absorbed from very dilute solutions in the soil is recognized, so it will be necessary to explore a similar range of concentration in the study of the question from a natural aspect. Obviously the use of solutions supplying phosphate at the rate of several hundred parts per million obscures the problem rather than elucidates it. In soil cultures it is impossible to evaluate any action of the roots on the solid phase, so the investigator must resort to solution cultures to obtain information concerning minimal concentrations and optimal concentrations. In these experiments the soil cultures proved valuable in indicating that, in the neutral soils used, the composition of the displaced solution gave the approximate composition of the physiological soil solution with respect to phosphate, and also in indicating the range of concentration which it would be profitable to study. Other investigators, PARKER and TIDMORE (11), VON WRANGELL and HAASE (17), and a host of others, have published data showing that soils in other parts of the world afford a similar range in concentration of phosphate in the soil solution.

The data here reported, obtained from both soil and solution cultures, indicate that one part per million of phosphate in the soil solution, if main-

TABLE VII

EXPERIMENTS 1, 2, AND 3

FLOWING SOLUTION CULTURES. ANALYSIS OF SAP EXPRESSED FROM TOPS OF WHEAT PLANTS GROWN IN CONSTANTLY FLOWING SOLUTION, AFTER FREEZING AT -15°C . TO -20°C . TO "KILL" THE TISSUE

CULTURE NUMBER	EXPERIMENT 1 (June 14th to July 23rd)					EXPERIMENT 2 (June 24th to September 8th)				EXPERIMENT 3 (September 8th to October 21st)			
	1 (CONTROL)	2	3	4		5	6	7		8	9	10	11
Culture solution pH.	5.5	7.0	5.5	7.0		5.5	5.5	5.5		5.5	7.0	5.5	5.5
PO ₄ ppm.	5.0	5.0	50.0	50.0		0.05	0.5	5.0		0.1	0.1	1.0	5.0
Sap analysis	6.26	6.18	6.13	6.28		6.54	6.30	6.29		6.40	6.24	6.29	6.20
PO ₄ (color) ppm.	2940.	2940.	3030.	2780.		800.	3300.	2800.		N.D.	570.	2700.	3450.
PO ₄ (total) ppm.	3040.	3030.	3030.	2870.		786.	3490.	2930.		810.	784.	3470.	4630.
SO ₄ ppm.										1070.	3670.*	890.	1370.
NO ₃ ppm.										N.D.	N.D.	5000.	5300.
Ca ppm.						720.	800.	673.		760.	1130.	715.	665.
Mg ppm.						700.	560.	645.		670.	980.	580.	590.
K ppm.												6200.	7600.
Fe ppm.										35.	43.	20.	19.
Ratio, moisture: dry matter.	5.1	5.5	5.3	5.0		4.8	6.2	5.2		3.9	4.2	5.1	5.0
Sap phosphates as per cent. dry matter	1.55	1.66	1.60	1.49		0.38	2.16	1.52		0.32	0.33	1.77	2.32
Total phosphate as per cent. dry matter													
Non-sap phosphate as per cent. dry matter	2.26	2.17	2.26	2.17		1.19	2.90	2.49		0.68	0.87	2.40	2.53
Ratio, sap phosphate: total phosphate	0.71	0.51	0.66	0.68		0.81	0.74	0.97		0.36	0.54	0.63	0.51
	0.69	0.77	0.71	0.69		0.32	0.74	0.61		0.47	0.38	0.74	0.92

tained, is adequate for the growth of Pusa 4 wheat under the conditions of the experiments.

A comparison of the results of soil and solution cultures (tables IV and VI) leads to the conclusion that, in these neutral, highly buffered soils, the displaced solution represents the physiological soil solution. Apparently the carbonic acid of the roots is neutralized immediately by calcium carbonate or other reactive components of the solid phase of the soil, preventing a change in the reaction sufficient to affect the solubility of the soil phosphates. This conclusion is supported by the results obtained from soil 33 in comparison with soil 33a (table IV). The addition of a considerable quantity of very finely ground rock phosphate which did not affect the soil solution appreciably, did not increase the absorption of phosphate by the wheat plants. Had carbonic acid played any appreciable part under these conditions the absorption would have been considerably augmented.

In the soil cultures, calcium in the soil solution is recognized as an agent active in the *retardation* of the solution of solid phase phosphate under a vigorously growing crop (table V). However, to account for the range in phosphate concentration in different soil types of the same reaction, it is necessary to consider other factors besides the concentration of calcium in the soil solution. That the nature of the solid phase phosphate is very important is indicated by the behavior of soil 33 (table III). The added phosphate was largely precipitated to form a solid phase compound yielding the same concentration of phosphate in the soil solution with or without a growing crop; and this concentration could not in any way be related to the concentration of phosphate in the original soil solution. This also did not vary under the action of a heavy crop.

It is permissible to study the results of the solution culture experimentation in greater detail. It must be admitted at the outset that the plants receiving 0.5 ppm. PO_4 , culture 6, experiment 2, table VI, were spindling and made growth similar in amounts to those plants receiving 0.05 ppm. PO_4 and 0.1 ppm. PO_4 . The composition, however, was comparable with the controls receiving 5.0 ppm. PO_4 . No explanation is apparent at present. Further experimentation is necessary in this range of concentration between 0.1 ppm. PO_4 and 1.0 ppm. PO_4 .

Other cultures indicated the same conclusion as did the soil cultures and substantiated the conclusion of SCHLOESING fils (13) referred to above. Below 1.0 ppm. PO_4 in the culture solution the absorption of phosphate by these plants was seriously affected. The composition (with respect to phosphate) of the expressed sap and the dry matter of the plants grown in solutions containing less than 0.5 ppm. PO_4 differed greatly from that of others receiving the more liberal concentrations. And the composition of

the plants, expressed in these terms, appeared to be approximately unaffected by the composition of the culture solution (with respect to phosphate) between 0.5 ppm. PO_4 and 50 ppm. PO_4 for the high level and between 0.5 ppm. PO_4 and 0.1 ppm. PO_4 for the low level. As regards total absorption per plant by the tops the same relationship holds except that 0.5 ppm. PO_4 in the culture solution allowed an absorption of phosphate intermediate between these groups.

What does this mean?

It seems apparent that the composition of the plant is affected by the composition of the soil solution, or culture solution, but there is no direct proportionality. Doubling the concentration in the solution does not cause a proportionate increase in absorption as 0.05 ppm. PO_4 gave results comparable with 0.1 ppm. PO_4 . More emphatic is the fact that 5.0 ppm. PO_4 (and even 1.0 ppm. PO_4) gave results strictly comparable with 50 ppm. PO_4 when one considers the amount of growth and tillering, the total phosphate absorbed, the percentage composition of the dry material, the composition of the expressed sap with respect to phosphate, and the buffer qualities of the sap. If there is a range of direct proportionality it is suggested that it will be limited to a relatively narrow range between 0.1 ppm. PO_4 and 1.0 ppm. PO_4 in the culture solution. It is significant that this is a range very common to the soil solution and is deserving of further attention.

Contributing factors of great importance need consideration and control. Varietal differences provide a field for exploitation and it is very possible that the results reported in this paper would be somewhat different if other varieties were used.

Different types of plants exist and are physiologically different in their behavior in soils. Will these differences be as apparent in solution cultures? Seasonal differences cannot be eliminated on a large scale at present. In this investigation similar controls were used in all three experiments involving the solution culture and a study of table VI shows that growth, the absorption, and the time taken to reach a given stage of maturity are dependent in some degree on seasonal conditions.

The question of the effect of phosphate on the "earliness" of wheat is raised by the results of the solution culture experiments. It is generally accepted that phosphate hastens maturity under field conditions. The figures in table VI do not support the field results for some reasons not yet understood. Various possibilities may be mentioned among which are varietal differences; greenhouse conditions *may* not be comparable with field conditions; the action of the phosphate in the field may be indirect and due to the changed *fertility* of the soil.

Criticism may be levelled at these experiments on account of the period of harvesting. Field workers agree that with cereals the period of "flowering" is the most reliable index of the period of ripening and is much easier to determine than is the stage called ripeness. The work of WIEBE (16) with oats may be cited in support of this contention.

Summary

1. Wheat plants were grown in soil cultures and in flowing solution cultures in an attempt to study the absorption of phosphate.

2. The composition of the soil solution, especially with regard to phosphate, was determined by analysis of the displaced solution. In the several soils studied the concentration varied from 0.15 ppm. PO_4 to about 10 ppm. PO_4 . The behavior of the phosphate under the influence of a crop of wheat was studied by comparison with uncropped controls.

3. Flowing solution cultures were used to maintain the concentration of phosphate. Concentrations from 0.05 ppm. PO_4 to 50 ppm. PO_4 were studied in a series of three experiments.

4. While the absorption of phosphate was affected by the concentration of the soil or culture solution with respect to phosphate, there was no direct proportionality.

5. The data indicate that there were two categories under the conditions of these experiments:

- (a) Vigorous growth with tillering and high absorption of phosphate between 1.0 ppm. PO_4 and 50 ppm. PO_4 in the culture solution.
- (b) Small growth with greatly reduced tillering and low absorption of phosphate between 0.05 ppm. PO_4 and 0.1 ppm. PO_4 in the culture solution.

The amount of growth of tillering, and quantity of phosphate absorbed by the plants in each group were remarkably similar.

6. If a region of direct proportionality exist, it is suggested that it will be in the range between 0.1 ppm. PO_4 , and 1.0 ppm. PO_4 —the range most commonly met with in soil solutions.

7. The composition and buffer qualities of the expressed sap were reflected by the total absorption of phosphate. Phosphate was effective in buffering against alkali, but the concentration of phosphate had no observable effect against acid. An excess of base in the expressed sap suggested the presence of an organic anion.

8. The non-sap phosphate of the plant showed no proportionality to the concentration of phosphate in the culture solution or in the expressed sap.

9. The displaced solution of the soils studied, neutral and highly buffered in character, represented the physiological soil solution with respect

to wheat. In these soils the concentration of phosphate in the soil solution was dependent on the nature of the solid phase rather than on the composition of the soil solution.

DEPARTMENT OF AGRICULTURE,
PERTH, WESTERN AUSTRALIA.

LITERATURE CITED

1. ANDERSSSEN, F. G. A device for maintaining constant level of culture solutions. *Plant Physiol.* 1: 417-418. 1926.
2. ATKINS, W. R. G. The rapid determination of available phosphate in soil by the coeruleo-molybdate reaction of Denigés. *Jour. Agr. Sci.* 14: 192-197. 1924.
3. BEYER, H. Bericht über die im Sommer 1867 an der Versuchs-Station Regenwalde ausgeführten Wasserculturversuche. *Landw. Vers. Stat.* 11: 262-287. 1869.
4. BIRNER, DR., and LUCANUS, DR. Wasserculturversuche mit Hafer. *Landw. Vers. Stat.* 8: 128-177. 1866.
5. BREAZEALE, J. F., and BURGESS, P. S. The availability of phosphate in calcareous or alkaline soils. *Arizona Agr. Exp. Sta. Tech. Bull.* 10. 1926.
6. BURD, J. S., and MARTIN, J. C. Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265-295. 1923.
7. HOAGLAND, D. R., and MARTIN, J. C. A comparison of sand and solution cultures with soils as media for plant growth. *Soil Sci.* 16: 367-388. 1923.
8. ————. Effect of salts on the intake of inorganic elements and on the buffer system of the plant. *Calif. Agr. Exp. Sta. Tech. Paper* 8. 1923.
9. KOSSOWITCH, P. Le rôle des plantes dans la dissolution des principes nutritifs du sol qui se trouvent à l'état nondissous. *Ann. de la Sci. Agron.* 2nd series, I: 220-245. 1903.
10. NEWTON, J. D. A comparison of the absorption of inorganic elements, and of the buffer systems of legumes and non-legumes, and its bearing upon existing theories. *Soil Sci.* 15: 181-204. 1923.
11. PARKER, F. W., and TIDMORE, J. W. The influence of lime and phosphatic fertilizers on the phosphorus content of the soil solution and of soil extracts. *Soil Sci.* 21: 425-441. 1926.
12. PRIANISCHNIKOW, D. Über den Einfluss von kohlensäuren Kalk auf die Wirkung von verschiedenen Phosphaten. *Landw. Vers. Stat.* 75: 357-376. 1911.

13. SCHLOESING fils, TH. Etude sur l'acide phosphorique dissous par les eaux du sol. *Ann. de la Sci. Agron.* **1**: 316-359. 1899.
14. SCHMIDT, C. L. A., and HOAGLAND, D. R. Table of pH, H^+ and OH^- values corresponding to electromotive forces determined in hydrogen electrode measurements, with a bibliography. Univ. of California, Pub. in Physiology, **5**: 23-69. 1919.
15. TEAKLE, L. J. H. Phosphate in the soil solution as affected by reaction and cation concentration. *Soil Sci.* **25**: 143-162. 1928.
16. WIEBE, G. A. Inheritance of characters in a barley and an oat cross. Master's Thesis, Div. Genetics, Univ. of California. 1925.
17. VON WRANGELL, M., and HAASE, W. Über den Phosphorsäuregehalt natürlicher Bodenlösungen. *Landw. Jahrb. (Sonderabdruck. Zeitschr. f. wissenschaft. Landwirtsch.)* **63**: 707-738. 1926.

THE EXTRACTION OF NITROGENOUS MATERIALS FROM PEAR TISSUES

F. B. LINCOLN AND A. S. MULAY

Three problems have been investigated in connection with removing the nitrogenous compounds from pear tissues: first, a suitable method for preservation of tissues previous to such an extraction; second, methods for obtaining an aqueous extract; third, the nitrogenous fractions found in expressed sap. In general the total N extracted has been divided into proteins and non-proteins, which because of the lack of exact separation are referred to as "proteins" and "non-proteins." Apple and spinach tissues were also included in the investigation for the purpose of comparison. As it was not possible to demonstrate the presence of nitrates in such pear tissues as were used, the simple Gunning method for the determination of total nitrogen of the A. O. A. C. was used in all cases. For the sake of brevity reference has been made to previous work in this field only when it seemed necessary.

I. The influence of preservation on distribution and extractability of nitrogenous materials of pear tissues

The treatments investigated were of two types: (1) those which kill—such as desiccation, freezing, and the use of chemical cytolizing agents; (2) treatments permitting the tissues to remain alive, such as moderately low temperatures. Besides these treatments, which were intended to minimize chemical change, others were tried which were considered not to be so favorable. These latter were intended to show the influence of two disturbing factors, autolysis, and heat coagulation of the proteins. The several maltreatments used were the storage of minced tissues, the incubation of such tissues, and the exposure to coagulating temperatures during desiccation.

MATERIALS AND METHODS

The leaves used were of the Beurre Hardy variety collected in the early summer. The other tissues were bark and wood from the basal portion of vigorous-growing Bartlett shoots. The investigation covered a period of two years. The second year the same tissues were used but the N content of all was higher because the samples were taken from younger trees. The leaves for 1928 appeared to contain more N because the petioles were not included. Usually bark and wood were separated from each other at the beginning of the various treatments. In one case the twigs were frozen with the bark on the wood, and the separation made after thawing.

DESICCATION

The general method employed was to subject a sample of tissue to some preservation treatment and then to measure the influence of the treatment by its effect on the "non-proteins" and the total extractable nitrogen. The procedure followed in obtaining these fractions will be reported later in this paper.

Samples were dried at two temperatures, 50° and 85° C. These temperatures were chosen because THOMAS (7) and others had found the former

TABLE I

PEAR LEAVES, 1927

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLE
TOTAL NITROGEN = 1.94 PER CENT. OF DRY WEIGHT

LOT	TREATMENT	TOTAL N IN FILTRATE "NON-PRO- TEIN"	TOTAL N IN IRON PRECIP- ITATE "PRO- TEINS"	TOTAL N EXTRACTED
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
L 1	Fresh	3.4	25.5	28.9
	Fresh	3.8	29.0	32.8
L 2	Dried 50° C.	5.05	22.1	27.15
	Dried 50° C.	4.4	25.6	30.0
L 3	0° C. 1 week	3.18	29.8	32.98
L 4	4-5° C. 1 week	3.26	30.2	33.46
L 5	4-5° C. 4 weeks	6.58	30.4	36.98
L 6	7° C. 2 weeks	3.48	21.1	24.58
L 7	4-5° C. 1 week (minced)	2.48	15.15	17.63
L 8	Minced, kept at room temp. for 24 hours and then dried at 50° C.	5.15	14.9	20.05
L 9	-23° C. 8 weeks	3.6	28.9	32.5
L10	Frozen (liquid air)	2.57	16.9	19.47
L11	Ether	3.16	10.12	13.28
L12	Incubated 37° C. 4 days	4.86	7.84	12.75

PEAR LEAVES, 1928

TOTAL NITROGEN = 2.808 PER CENT. OF DRY WEIGHT

		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
L13	Fresh	3.4	37.58	40.98
L14	Minced. Incubated 24 hrs.	3.4	20.7	24.10
L15	Dried at 50° C.	5.0	42.5	47.50
L16	Dried at 85° C.	3.58	3.50	7.08

to be quite satisfactory, and the latter was supposed to be high enough to show the injurious effects of temperature. Fan ventilated ovens were used and even at 50° C. most of the moisture was removed in the first three hours. It may be stated that moisture determinations made at 50° C. on these materials were found quite reliable; with an increase in temperature to 100° only about an additional 1 per cent. of moisture was given off.

From the data presented in tables I, II, and III, the influence of drying at 50° C. may be seen by comparing the data from desiccated and fresh samples (L2, L15, B2, B11, W2, W12). In leaves, drying increased the filtrate nitrogen, the "protein" extracted being about the same as that

TABLE II

BARK, 1927

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLE
TOTAL NITROGEN = 0.8008 PER CENT. OF DRY WEIGHT

LOT	TREATMENT	TOTAL N IN FILTRATE "NON-PRO- TEIN"	TOTAL N IN IRON PRECIP- ITATE "PRO- TEINS"	TOTAL N EXTRACTED
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
B 1	Fresh	8.63	11.15	19.78
B 2	Dried at 50° C.	8.73	16.35	25.08
B 3	-23° C. 1 week	7.1	8.63	15.73
B 4	-23° C. 1 week (un- separated from wood)	10.29	10.29	20.58

BARK, 1928

TOTAL NITROGEN = 1.022 PER CENT. OF DRY WEIGHT

		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
B 5	Fresh	8.55	17.35	25.9
B 6	Frozen and thawed 24 hrs.	10.12	13.78	23.9
B 7	Unseparated from wood frozen	13.8	21.6	35.4
B 8	Ether	7.3	15.8	23.1
B 9	Minced and kept for 24 hours, 20° C.	5.8	21.3	27.1
B10	Minced and kept for 24 hours, 20° C., then dried at 50° C.	4.64	1.38	6.02
B11	Dried at 50° C.	8.6	22.1	30.7
B12	Dried at 85° C.	9.54	9.86	19.4

TABLE III

WOOD, 1927

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLE
TOTAL NITROGEN = 0.539 PER CENT. OF DRY WEIGHT

LOT	TREATMENT	TOTAL N IN FILTRATE "NON-PRO- TEIN"	TOTAL N IN IRON PRECIPI- TATE "PRO- TEINS"	TOTAL N EXTRACTED
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
W 1	Fresh	14.5	8.63	23.13
W 2	Dried at 50° C.	15.65	8.00	23.65
W 3	- 23° C. 1 week	15.2	10.63	25.83
W 4	- 23° C. 1 week (un- separated)	16.6	10.4	27.00
W 5	Minced—incubated at 37° C. for 4 days	18.85	11.1	29.95

WOOD, 1928

TOTAL NITROGEN = 0.868 PER CENT. OF DRY WEIGHT

		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
W 6	Fresh	16.2	12.2	28.4
W 7	Frozen and thawed for 24 hours	17.4	18.4	35.8
W 8	Frozen unseparated from bark	17.1	20.0	37.1
W 9	Ether	16.4	19.7	36.1
W10	Minced and kept 24 hours at 20° C.	10.9	19.7	30.6
W11	Minced and kept 24 hours at 20° C. then dried at 50° C.	12.5	3.0	15.5
W12	Dried at 50° C.	16.4	17.8	34.2
W13	Dried at 85° C.	15.3	11.1	26.4

from the fresh sample. In wood the difference was not so marked, and in bark the yields were about the same for both treatments. The results showed that drying bark and wood at 50° C. was very satisfactory. Why this treatment apparently caused hydrolysis of proteins in the leaves, as evidenced by the increase in the "non-protein" fraction, is not known.

The temperature of 85° C. (L16, B12, W13), as was expected, reduced the amount of "protein" extracted. This effect was evident in all the extracts, with a striking reduction in the leaf and bark samples. The higher temperature seemed to give a truer picture of the "non-protein" N in leaves than did drying at 50° C. while the same fractions in bark and wood were influenced slightly in the direction opposite to each other.

The use of 85° may have its place in experiments where only total N and "non-protein" fractions are under consideration. It has the advantage that killing and dehydration are more rapid.

Desiccation must have favor in cases of long periods of storage and, what is also very important, dried samples of wood tissues allow easier disintegration by grinding, and also permit the use of sieves for the grading of the ground materials.

FREEZING

Freezing is often a very convenient method for rapidly limiting chemical changes, particularly when samples are to have juices expressed from them. The samples in this experiment were placed in glass containers and were stored for varying lengths of time at -27° C. in a cold storage room. In this way leaves were kept in an unaltered condition for 8 weeks (L9). They retained their green color until the sample was thawed. Bark and wood held at this temperature for one week showed only slight changes (B3, W3). Freezing may have a disadvantage in that the tissue is left dead and at the same time full of water, which may permit many changes to take place upon thawing. NIGHTINGALE, ROBBINS, and SCHERMERHORN (5) report changes in nitrogen fractions of sweet potato roots due to thawing at 2° C. for 12 hours. To get the full effect of the possible changes during and following thawing, wood and bark samples which had been frozen were allowed to stand in their containers at room temperature for 24 hours before they were ground and extracted (B6, W7). While this treatment is extreme, the changes effected in the N fraction here determined were surprisingly small in the wood, but in the bark some hydrolysis had occurred, yet not so much as was expected.

Delaying the separation of bark from the wood until after thawing of the tissues was tried both years, with similar results (B4, B7, W4, W8), an increase in all nitrogen fractions. These results appear to be similar to those obtained with frozen samples subjected to a long period of thawing (B6, W7) rather than to separate samples of frozen bark and wood, which were ground and extracted without a long period of thawing. As the time required for the separation of the bark from the wood of the frozen samples was very short, there is no explanation to offer for this resemblance. During freezing and thawing there is a movement of water out of the sample to its surface and then back again to the interior. How much this translocation of water is accompanied by the movement of solutes is not known.

COLD NON-FREEZING TEMPERATURES

Moderately cold temperatures, 0° C. to 7° C., preserved fresh leaves well for a week or two (L3, L4, L6). Holding at 4° C. (L5) for a month resulted in an increase of "non-protein" N. Even after this long period

the sample was free from mold and had retained its full green color. Preservation at these temperatures has the advantage of keeping the tissue alive and at the same time greatly reducing chemical changes.

CHEMICAL REAGENTS

Ether has long been used to make cells permeable, so that their liquid contents could be more easily extracted. This reagent seemed to affect the extractable protein fraction of different tissues differently; there was a marked reduction in leaves (L11), slight reductions in bark (B8), and an increase in the wood (W9). In the same samples ether treatment did not greatly influence the "non-protein" fraction although there may have been a reduction of it in the bark. The ether treatment was not exactly alike in the various samples. The leaves were immersed in ether, while with bark and wood 25 ml. of ether was placed in a quart jar and only the vapor came in contact with most of the tissue.

Liquid air has been effective in other studies in making tissues permeable. In this experiment (L10) it reduced both nitrogen fractions in the treated leaves.

MALTREATMENTS

EL SAWY (3) has reported the effects of injury to pear tissues resulting from cutting shoots into short lengths. The effect was a rapid hydrolysis of polysaccharides accompanied by an increase in respiration. In the present investigation several treatments were made in which the tissues were minced in a food chopper. The minced tissues were then kept at room temperature for a day in closed glass containers. As a result of this severe treatment leaves (L14) showed a decrease in extractable "proteins." Bark and wood (B9, W10) acted alike, but contrary to leaves; they showed a decrease in the "non-protein" fraction and an increase in proteins. When portions of the minced tissue were dried at 50° C. (L8, B10, W11) there was a great reduction of extractable "protein" N.

A sample of minced leaves was kept for a week at 4° C. (L7) resulting in a marked reduction of both fractions of extractable N. It is not clear why injury should so often be accompanied by a reduction in "non-protein" N. This sample was tested for free ammonia and no volatile alkali was found.

When leaves and wood were minced and suspended in water, covered with toluol, and incubated at 37° C. for 4 days, some slight hydrolysis took place (L12, W5). This change was very much less than was expected, assuming that the conditions were quite favorable for autolysis. These experiments, taken with those where a long period of thawing was used (B6, W7), give some idea of the effects of enzymes present in the tissue when allowed to act for a moderate period. They are very feeble when

compared with the effects of added animal enzymes. Some data showing the effect of enzymes present in leaf tissue are given by CHIBNALL (1). From these data it appears that their action may last for only a short time.

The effect of maltreatment by overheating of "proteins" is found in the samples dehydrated at 85° C. With leaves and bark (L16, B12) the "protein" fraction was markedly reduced in extracts of heated samples.

APPLE TISSUES

Apple tissues of McIntosh variety were collected in the middle of the summer and subjected to the treatment while fresh. The results are found in table IV. It will be seen that the N fractions extracted from the different tissues are in the same relation to each other as they are in the extracts of pear tissues. The striking difference is that much more of the N of apple tissues is extracted from each of the various tissues.

TABLE IV

APPLE TISSUES

ALL VALUES ARE EXPRESSED AS PER CENT. OF TOTAL N IN THE SAMPLES

TREATMENT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN IRON PRECIPITATE "PROTEINS"	TOTAL N IN EXTRACT
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Fresh leaves	6.65	36.65	43.3
Fresh bark	12.12	32.78	44.9
Fresh wood	35.9	13.7	49.6

When preservation must be resorted to there are several procedures which will give results quite similar to fresh tissues. These are dehydration at 50–55° C., storage at 0° C. or a few degrees above for short periods, or freezing when a longer storage period is necessary. If the "protein" fraction is not to be extracted, drying at temperatures of 80–85° C. will stop changes rapidly. Samples should be preserved with as little mincing as possible since injured tissue may change rapidly.

How best to preserve plant material for chemical analysis is still an open question. The answer to this question depends somewhat on the investigation and the equipment available for it. Facilitation of work may be of more importance than extreme exactness; and if fresh material can not be used, circumstances will determine the method of preservation. Thus, if storage is to be for a long time or if firm tissues must be disintegrated, drying is likely to be the best method. Low temperatures have their place where sap concentrations are of chief interest. It should be

remembered that different parts of a plant may not all preserve equally well by a given method and also that any organ may so change its composition or nature during the season that the same method may not give equally reliable results at all times. The present study aims to show the types and magnitudes of changes that may be introduced by preservation, and will help to determine the dependability of results obtained by the different methods.

II. Methods for the extraction of cytoplasmic nitrogen

Generally only 10–25 per cent. of the total N of pear tissues is soluble in the expressed sap, and is almost wholly in a “non-protein” form. The present study was undertaken to see how much of the remaining insoluble N could be made soluble or peptized in water. The procedure was to extract the very finely divided tissue with water containing different ions, or to hydrolyze with acid or animal enzymes. There are at least three disturbing factors to such extractions: (1) the acidity of the tissue, (2) naturally contained tannins, and (3) the inability to get the tissues absolutely disintegrated. The latter is best accomplished by drying the tissues at 50° C., grinding them coarsely in a mill and completing the grinding to a powder in a ball mill. When such leaf powder was examined under the microscope it was found that most of the cells were completely broken apart. Only a few bits of tissue of several cells each remained; these were portions of the harder tissues composed of smaller cells.

There is considerable uncertainty regarding the action of the tannins present in pear tissues. They seem to be present; for expressed sap will cause precipitation in a gelatin solution, and will also give the color reactions of tannins. However, when tannic acid was added to water extracts of pear tissues, table VIII, it did not increase the amount of N in the precipitate over the amount caused by the acid used to adjust the reaction for the precipitation by tannins. Tannin-protein complexes are soluble in alkaline solutions, which may account for the peptizing effect of alkaline solutions in these studies. On the other hand, spinach, which very likely contains no tannins, responds like pear tissues when extracted with alkaline solutions. As the work proceeds and attempts are made to purify cytoplasmic proteins perhaps some evidence of a tannin residue may appear. There is also the possibility that the inherent tannins do not precipitate cytoplasmic proteins. The extracts of pear tissues were in themselves so highly colored that it was not possible to apply tannin color reactions to them. Furthermore, combined tannins would not give the test had they been present. There was also the hope that in alkaline solutions the tannins would be oxidized by the air which was forced through the solutions by the mechanical stirrer, and in their oxidized form would not combine with the

proteins when the solutions were neutralized. The solutions did become dark in color, but the fate of the tannins is not known.

Before the tissues can be subjected to a water extraction they should be disintegrated as completely as possible. This was accomplished by two methods. In the one the fresh tissue was ground with sand and water, and in the other the tissue was desiccated, ground, and then the powder extracted with water. The first method was used in investigating the various procedures for preserving tissue reported previously in this paper. This was required because some of the samples had to be handled in the succulent condition for comparison with the dehydrated ones.

The sand grinding method for fresh material is as follows: All samples, fresh, frozen, and dried, were ground with water, sodium carbonate, and sand, in a "Quaker City" corn mill. This mill is similar in principle to the Enterprise "Nixtamal" mill mentioned by several investigators. Generally the materials were minced in a food grinder or a Wiley mill before grinding with sand and sodium carbonate. The procedure was to weigh out 200 grams of the minced sample, and add to it 25 grams of sodium carbonate and 150 or more grams of sand. These were ground together with water. Grinding was most effective when the sample was kept in a non-liquid pasty mass. The higher yields of precipitated N in 1928 were the result of using less water while the samples were being disintegrated. Grinding four times in the mill was generally sufficient to reduce the materials so that about 75 per cent. of the sample would pass through a 100 mesh sieve.

When grinding was complete, the material was stirred into about 1500 ml. of water, allowed to stand for an hour, and then separated from this water by centrifuging. The solid residue was washed with water four times and the extracts combined. The extracts were treated with toluol and kept at 0° C. The time required for grinding, mixing, and centrifuging a sample was about three hours. It is believed that the amount of precipitable N extracted can be increased over the values here reported by vigorously agitating the ground material in water for 30 minutes or so before centrifuging. The residue after washing was dried at 85° C. and again ground for sampling. Total nitrogen was determined in triplicate on the extract and the residue.

The second method, using dry powder, is much more exact because the tissue can be disintegrated more completely and can be graded by the use of sieves and, wherever it can be used, it is recommended as superior to the previous method. It was used in attempts to get complete extraction of nitrogen, the results of which will be given further on in this paper. The method is essentially to suspend 10 grams of dry pulverized tissue in 250 ml.

of extracting solution, the suspension being agitated by a mechanical stirrer for two hours. At the end, the extracted tissue was collected by centrifuging. Table V gives the influence of temperature on extracting leaf powder with water. The time of these extractions was two hours.

TABLE V

EFFECTS OF TEMPERATURE ON EXTRACTION OF NITROGEN FROM LEAF POWDER IN WATER

TEMPERATURE	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
20° C.	17.45	6.15	11.3
50° C.	14.9	6.76	8.14
80° C.	10.8	8.65	2.15

The duration of the extraction has its influence, as may be seen from table VI, which presents the results of extraction of leaf powder with water made alkaline with sodium carbonate. Two hours was the time selected for all subsequent extractions.

TABLE VI

EFFECTS OF LENGTH OF TIME OF EXTRACTION ON THE NITROGEN FRACTIONS OF LEAF POWDER + Na_2CO_3

TREATMENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
2 hours, no stirring	27.80	5.2	22.6
30 minutes, stirring	43.1	5.7	37.4
2 hours, stirring	47.50	5.0	42.50
Residue from 2 hours, stirred a second 2 hours	3.0

PROTEIN AND NON-PROTEIN NITROGEN

While the interest here is to see how much of the ordinarily insoluble N of pear tissues could be peptized and extracted by various means, it is also worth knowing how much hydrolysis had accompanied each of the treatments. It was assumed that an increase in the "non-protein" N would be a measure of hydrolysis. This made it necessary to fractionate the N of the extracts into "protein" and "non-protein." A good discussion of the

separation of proteins from their hydrolytic products is given by THOMAS (8). He concludes that colloidal iron effects such a separation.

TABLE VII

PRECIPITATION VALUES ON FRESH LEAF AND FROZEN UNSEPARATED BARK AND WOOD, 1927,
EXPRESSED AS PERCENTAGE OF TOTAL NITROGEN

Na ₂ CO ₃ EXTRACT	TOTAL N IN EXTRACT	HEAT COAGU- LATION	IRON PRECIPI- TATE	ACID PRECIPI- TATE, pH 1	TOTAL PRECIPI- TATED	ACID pH 1 ON ORIGINAL
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Leaf	29.0	3.24	5.19	14.8	23.23	25.5
Bark	21.3	9.87	5.94	3.56	19.37	10.65
Wood	24.5	5.3	6.5	2.39	14.19	9.45

In order to find out the amount of nitrogenous compounds which was precipitated from pear extracts by various means, some experiments were made using sodium carbonate extracts from fresh leaves, from bark, and from wood. The bark and wood had been preserved by freezing and were not separated from each other until thawed. These extracts were brought to pH 5, near the isoelectric point of many proteins, by the addition of hydrochloric acid. The acidity was estimated by indicator papers. These acidified extracts were boiled and the heat-coagulated materials collected. The liquid residue was treated with colloidal iron. The precipitate formed was collected and the liquid residue was further acidified to pH 1 and a third precipitate was obtained. The results of these fractional precipitations as well as the total amount of N precipitated are reported in table VII. Portions of the original extracts were made acid to pH 1 and a precipitate was obtained, the N content of which is reported in the last section of table VII. From this data it appears that heating, or use of colloidal iron removes N compounds from the extracts of wood and bark, which acid (pH 1) alone does not remove. It may be suggested that acid alone in this case does not precipitate the heat coagulated fraction. The same appears to be true for spinach, which is discussed later. The heating of bark extracts gave a very large volume of jelly which was only freed with difficulty from its soluble forms of N.

Further experiments were carried out in 1928 in which alcohol, heating, strong acid, and tannin are compared as precipitating agents. The results are presented in table VIII.

Here the amount of N precipitated by 65 per cent. alcohol compares very well with that brought down by strong acid (pH 1). However, in leaves and wood the acid is more inclusive. To use tannic acid the extract must

TABLE VIII

PER CENT. OF TOTAL N PRECIPITATED BY HEAT, ACID, AND ALCOHOL

TISSUE	TOTAL N IN EXTRACT	ALCOHOL 65 PER CENT.	HEAT PH 6	PH 4	PH 4 + TANNIN	ACID PH 1
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Fresh wood	28.4	12.1	4.15	5.8	5.5	12.2
“ bark	25.9	16.7	11.08	16.5	14.7	17.35
“ leaves	40.98	22.95	8.78	11.9	8.7	37.58
“ spinach	89.2	55.9	2.2			57.4
Root wood (dry).....	76.5	16.2				19.4

be made more acid, pH 4, for the precipitation of proteins (7). Why the precipitation was not more complete when tannic acid was used is not

TABLE IX

INFLUENCE OF PH SERIES ON THE EXTRACTION OF NITROGEN FROM LEAF POWDER

ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLE

PH	REAGENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE “NON-PROTEIN”	TOTAL N IN PRECIPITATE “PROTEIN”
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	N/10 H ₂ SO ₄	15.8	7.7	8.1
5	Water	17.45	5.95	11.50
6	N/10 NaOH	19.35	5.65	13.70
7	N/10 NaOH	22.4	4.6	17.8
9	N/10 NaOH	28.85	3.7	25.15
12	0.2N Na ₂ CO ₃	47.5	5.0	42.5
13 +	2N NaOH	89.35	18.5	70.85

ROOT WOOD SERIES

INFLUENCE OF PH SERIES ON THE EXTRACTION OF NITROGEN FROM ROOT WOOD

TOTAL NITROGEN = 0.414 PER CENT. OF DRY WEIGHT

ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLE

PH	REAGENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE “NON-PROTEIN”	TOTAL N IN PRECIPITATE “PROTEIN”
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	H ₂ SO ₄	56.4	56.4	
5	Water	38.85	38.85	
12	0.2N Na ₂ CO ₃	57.8	56.8	1.00
13 +	2N NaOH	76.5	57.1	19.4

known. Iron is not effective without acidifying the extract, and to get a good yield the acidity must be increased to such an extent in these extracts that the acid alone becomes the precipitating agent. From the present study it appears that acid (pH 1) is a very suitable precipitating agent and probably gives a fair measure of the colloidal proteins contained in the extracts. The question as to the purity of the precipitate is left for future investigation.

Spinach has been included in this investigation in order that the results obtained with pear tissues could be compared with the results obtained by others who have worked principally with annual plants, and plants free from tannins. OSBORNE and WAKEMAN (6), working with a very different method of fractionation, report the colloidal proteins of spinach as being 58.7 per cent. of the total N, and the non-protein, proteose, and heat coagulable fractions as 33.5 per cent. These figures compare very favorably with the values here obtained by the use of strong acid as a fractionating agent; these values are 57.4 per cent. as acid precipitate, and 31.8 per cent. as filtrate N (table X).

TABLE X
SPINACH SERIES
TOTAL NITROGEN = 3.56 PER CENT. OF DRY WEIGHT

TREATMENT	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Fresh, H ₂ O	89.2	31.8	57.4
Dried, H ₂ SO ₄	42.0	42.0	
" , water	57.1	35.8	21.3
" K tartrate	48.8	34.6	14.2
" Na ₂ CO ₃	60.5	31.9	29.0
" " (duplicate)	60.9	27.6	32.9
" NaOH	84.0	35.8	48.2
" alcohol	23.3	23.3	

CHIBNALL and GROVER (2) reported "non-protein" N of spinach leaves as 26.7 per cent. which is about the same as was reported by OSBORNE and WAKEMAN (6). In both of these cases the heat coagulable N is not included, which fact probably accounts for the percentages being lower than for the filtrate after acid precipitation (here called "non-protein" N), which is 31.8 per cent. CHIBNALL and GROVER (2) and OSBORNE and WAKEMAN (6) report 37 per cent. of the N not extracted. In the present investigation, owing perhaps to better disintegration of the sample, only about 10 per cent. of the N remained unextracted. The method used on the fresh

spinach was the one already described with the exception that no sodium carbonate was used.

The "non-protein" N fraction was increased from 33.5 per cent. to 43.6 per cent. in the investigations of OSBORNE and WAKEMAN (6) by drying at 60° C. In the present study the increase caused by drying was less, being from 31.8 per cent. to 35.8 per cent., but at the same time the amount of extractable "protein" was reduced about two-thirds. OSBORNE and WAKEMAN (6) showed no such reduction and stated that dried material was equivalent to fresh. They determined their colloidal proteins by difference rather than by actual determination. That drying is effective in decreasing extractable "proteins" is confirmed by CHIBNALL's work on Runner beans (1) in which air drying decreased the extractable colloidal "proteins" about one-half and at the same time the soluble "non-protein" fraction changed from 24 per cent. to 39 per cent.

INFLUENCE OF HYDROXYL ION

Having set forth and discussed the procedure for the separation of "protein" from "non-protein" materials, we shall now return to the attempt to secure a more complete extraction of nitrogenous compounds.

The influence of alkalinity on the peptization of cytoplasmic proteins is shown in table IX. Here leaf and root wood powders were extracted with water and electrolytes chosen to give different hydroxyl ion concentrations. No effort was made to buffer the solutions. The reactions reported were determined by indicator papers as far as possible, the tests being made before and after agitation. The results show that alkalinity is a most important factor in extracting "proteins" from these tissues. Table X shows that the same is true for spinach tissue. Some hydrolysis took place in all tissues with the stronger concentrations of acid and base, the hydrolysis being very marked with strong sodium hydroxide acting on leaf powder. With spinach leaves sulphuric acid caused the most hydrolysis. It is not known what significance to attach to the fact that the lowest "non-protein" value (3.7 per cent.) occurs at pH 9 and that the value increases as the reaction goes in either direction, above or below pH 9. The reaction also has an important effect on the "non-protein" fraction in root wood and spinach.

SALT SERIES

GORTNER, HOFFMAN, and SINCLAIR (4) report a very interesting experience with the effect of neutral salts in the extraction of proteins from wheat flour. They found that the anions were very important and can be arranged according to effectiveness in the following order. $F < SO_4 < Cl < Tartrate < Br < I$. Of the cations they used, sodium and potassium were the least effective and calcium the most. Table XI shows the effect of some

neutral salts in 0.1 N concentrations on the extraction of "proteins" from pear leaves. The series from these results would be $\text{SO}_4 < \text{I} < \text{oxalate} < \text{tartrate}$. If it is assumed that potassium chloride would act similarly to sodium chloride then chloride would appear to be less effective than sulphate. In this study sodium was far better than calcium when they were used as chlorides. Calcium salts, even calcium hydroxide in a saturated solution, gave the poorest yields of any salts used. The effects of the ions of neutral salts are quite aside from the effects of hydroxyl ion concentrations, and are quite ineffective in peptizing cytoplasmic nitrogen when compared with the activity of hydroxyl ion.

TABLE XI

INFLUENCE OF SALT SERIES ON EXTRACTION OF NITROGEN FROM LEAF POWDER
ALL VALUES ARE IN TERMS OF PER CENT. OF TOTAL N IN THE SAMPLES

SALT USED, N/10	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
K Tartrate	24.05	6.05	18.00
K Oxalate	24.7	7.4	17.3
KI	22.0	6.2	15.8
K_2SO_4	20.0	5.9	14.1
NaCl	16.5	7.0	9.5
CaCl_2	7.75	6.3	1.45
$\text{Ca}(\text{OH})_2$	17.25	9.85	7.40

Tartrate, the best of the anions of neutral salts, when used in the extraction of N from spinach leaves was also quite inactive, when compared with the effectiveness of alkaline solutions. It is shown here that the anions of neutral salts differ from one another in their effectiveness to extract cytoplasmic proteins, but all of them are much inferior to hydroxyl ion in their yield of total extractable N. The ability or inability of various ions to peptize cytoplasmic proteins may through extensive study give an insight into some of the properties of the N constituents of protoplasm.

HYDROLYSIS

So far the best treatment for extraction of nitrogen had been 2N sodium hydroxide, which, in the case of leaf tissue, had extracted about 90 per cent. of the N. The question arose as to whether the extraction could be rendered more complete by means of hydrolysis. Proteolytic enzyme was obtained by the digestion of a fresh pig's stomach. The procedure was to digest 200 gm. of stomach in a liter of water at pH 2, for 24 hours at 45° C. For

digestion of a leaf sample, 15 ml. of this digestion liquid was used to 10 gm. of leaf powder in 250 ml. of water. The reaction and temperature used were the same as for the previous digestion. The hydrolysis was carried out for two periods of time, 2 hours, and 18 hours. Controls were run with pear tissue in water at pH 2 for the same periods of time. In the shorter period 63.5 per cent. of the N was made soluble, while the acid control for the same length of time gave 21 per cent. of the N in solution. In 18 hours the animal enzyme had rendered soluble 68 per cent. of the total N, and the acid control had done nearly as well, making 60.5 per cent. of N soluble. In the sample hydrolyzed for the shorter period it was found that not all of the sample had been thoroughly dispersed. It is quite possible that if it had not been for this mishap, hydrolysis would have proceeded as far in 2 hours as it did in 18 hours.

It is interesting that the acid control for 18 hours yielded nearly as much soluble N as did the animal enzyme. This may indicate activity of the leaf enzymes, for the hydrogen ion concentration in itself was hardly sufficient to accomplish such hydrolysis at the temperature used.

An acid hydrolysis with 18 per cent. hydrochloric acid for 24 hours on a steam bath made 75.6 per cent. of the N soluble, which is slightly better than the results with the animal enzyme.

Practically complete extraction of leaf N was accomplished by first extracting with 70 per cent. hot alcohol containing 1 per cent. acid, which gave 74.9 per cent. soluble N. This was followed by a sodium hydroxide extraction for three days at 40° C. which yielded a further 23.5 per cent., making the total N extracted 98.4 per cent.

Table XII gives an idea of the extractability of the nitrogenous compounds of the different tissues with 0.2 N sodium carbonate solution.

TABLE XII
TOTAL NITROGEN IN Na_2CO_3 EXTRACT OF SAMPLES DRIED AT 50° C.

TISSUE	TOTAL N IN EXTRACT	TOTAL N IN FILTRATE "NON-PROTEIN"	TOTAL N IN PRECIPITATE "PROTEIN"
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Leaves	47.50	5.0	42.50
Bark	30.7	8.6	22.1
Wood	34.2	16.4	17.8
Root wood	57.8	56.8	1.0
Spinach	60.9	31.9	29.0

III Distribution of nitrogen in expressed sap

In table XII it is seen that the different tissues of the pear tree, namely, leaves, shoot wood and bark, and root wood, are very different in their N contents and are strikingly unlike in the distribution of their "protein" and "non-protein" fractions. The difference between these tissues in their "non-protein" N would lead one to believe that the cells of these tissues must be functioning with quite different concentrations of the materials that go to make up the "non-protein" fraction. The opportunity to see what might constitute some of these differences was offered by the materials remaining from another investigation, which had required the collection of samples during the winter, and from which sap was expressed by a hydraulic press. Each time that an excess sap was obtained it was saved and preserved at 0° C. with chloroform. The composites thus accumulated during the winter were analyzed for their N fractions. The sap was from bark and wood of the tops and roots of vigorous-growing six-year-old Bartlett pear trees.

The N precipitated by colloidal iron was assumed to be protein N. The filtrate from the iron precipitation was precipitated with phosphotungstic acid. The N thus precipitated when compared with a similar precipitation of a protein free sample subjected to acid hydrolysis with 20 per cent. hydrochloric acid, gave the polypeptide N. The phosphotungstic acid precipitate after the acid hydrolysis gave the diamino acids. Mono-amino acid N was obtained by the usual VAN SLYKE method. Amide N was determined by hydrolyzing the filtrate from phosphotungstic acid precipitates for 2 hours with 4 per cent. HCl and distilling of ammonia after making the solution alkaline with magnesium oxide.

TABLE XIII

DISTRIBUTION OF NITROGEN IN EXPRESSED SAP
ALL VALUES ARE IN TERMS OF PERCENTAGE TOTAL N IN SAP

TISSUES	TOTAL CC. N/10 ACID PER 100 CC. OF SAP	PROTEIN N	POLY- PEPTIDE N	DIAMINO N	MONO- AMINO N	AMIDE N	RESIDUAL N
	cc.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Top bark .	49.5	4.25	16.77	2.63	45.0	17.6	13.7
Top wood..	154.5	4.30	64.80	6.60	6.2	3.1	15.2
Root bark...	42.5	4.95	33.92	1.88	31.6	9.9	17.9
Root wood..	154.5	5.25	49.99	3.11	19.7	3.9	18.0

The data in table XIII show that the wood contained three times as much of its N in the sap as in the bark. The amount of protein is relatively small and quite similar in the sap of all the tissues. Nearly half of the N of wood sap is in the form of polypeptides, and the actual amounts in this tissue are very large for more of the wood N is in the expressed sap. The polypeptide N may amount to 25 per cent. of the total N of wood. The quantity of diamino acids is small.

The bark while low in polypeptide and diamino N is very high in mono-amino acids; and this may be the highest of all bark N fractions. The bark is also highest in amides, particularly the top bark. The relatively small figures for residual N show that the fractionation has been quite complete. The methods of fractionation are standard and the differences so marked that some positive differences in composition are here indicated.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. CHIBNALL, A. C. The effect of low temperature drying on the distribution of nitrogen in the leaves of the runner bean. *Biochem. Jour.* **16**: 599-607. 1922.
2. ———, and GROVER, C. E. A study of leaf cell cytoplasm. I. The soluble proteins. *Biochem. Jour.* **20**: 108-118. 1926.
3. EL SAWY, A. H. Some effects of wounding on respiration and food reserves in the pear tree (*Pyrus communis*). Unpublished dissertation. University of California. 1927.
4. GORTNER, R. A., HOFFMAN, W. F., and SINCLAIR, W. B. Physico-chemical studies on proteins. III. Proteins and the lyotropic series. *Colloid Symposium Monograph* **5**: 179-198. 1927.
5. NIGHTINGALE, G. T., ROBBINS, W. R., and SCHERMERHORN, L. G. Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. *New Jersey Agr. Exp. Sta. Bull.* **448**. 1927.
6. OSBORNE, T. B., and WAKEMAN, A. J. The proteins of green leaves. I. Spinach leaves. *Jour. Biol. Chem.* **42**: 1-26. 1920.
7. THOMAS, ARTHUR W., and FRIEDEN, ALEXANDER. The gelatin-tannin reaction. *Jour. Ind. Eng. Chem.* **15**: 839-841. 1923.
8. THOMAS, WALTER. Nitrogenous metabolism of *Pyrus malus*, L. *Plant Physiol.* **2**: 55-66. 1927.

HEMICELLULOSE AS A STORAGE CARBOHYDRATE IN WOODY PLANTS, WITH SPECIAL REFERENCE TO THE APPLE

A. E. MURNEEK

(WITH FIVE FIGURES)

After the removal of sugars and starch by commonly accepted methods from properly prepared woody plant tissues, there usually remains a residue. A large part of this residual cell wall material may be hydrolyzed by dilute mineral acids to monosaccharides or other carbohydrates having the power to reduce copper compounds. Hence the quantity of this "digestible" fraction may be readily estimated. Such higher carbohydrates, other than starch, have been variously designated in literature as "polysaccharides," "acid hydrolyzable material," etc. More often, however, the name "hemicellulose" has been assigned to this group of complex compounds.

In most lignified tissues the relative amount of hemicellulose is very high. Moreover, in certain organs at least, it shows a marked seasonal fluctuation. Hemicellulose, therefore, may be readily available to the plant, possibly through enzymatic action. What is the significance of this cellular aggregation in the physiology of woody plants? Most certainly it can not be ignored in studies of carbohydrate distribution, since more often than not it constitutes the preponderant volume of the more labile forms of these substances.

It is the object of this paper to review briefly our present knowledge of the occurrence, distribution, and chemistry of hemicellulose in some higher plants. It is hoped that thereby further interest may be created in this highly important group of reserve carbohydrates.

Historical retrospect

There is reliable evidence (20) that as early as 1862 SACHS observed that upon germination a portion of the cell walls of the date seed disappears, leaving a much thinner permanent layer. Since then it has been shown by many investigators that a storage cellulose of this character is present in various parts of seeds of many plants, but especially in certain kinds of endosperms and cotyledons. After extensive observations and careful chemical studies, REISS, 1889 (*loc. cit.*) came to the conclusion that this labile cellular thickening is not a true cellulose and hence it should be definitely designated by the already suggested names of "amyloid" or "reserve cellulose." Two years later (1891) SCHULZE (26) proposed the name "hemicellulose" for all such substances of the plant cell wall, which upon

hydrolysis with weak mineral acids, usually give such sugars as galactose, mannose, arabinose, xylose, etc., thus clearly distinguishing it from the more fibrous cellulose, which is hydrolyzable only by strong acids and yields in most cases but glucose.

That hemicellulose is present in other tissues than those intimately associated with the embryo has been demonstrated by several European investigators but especially by DU SABLON (5) and SCHELLENBERG (23). Using as the material a number of typical woody plants, the former has shown by chemical analysis that hemicellulose accumulates in the fall, reaching a maximum in October in various vegetative organs, but particularly in twigs and roots. It is mobilized and reabsorbed during the following spring and usually shows a minimum concentration in summer (May-June). Microscopic observation of willow twigs collected in February showed clearly an incrustation interior to the cell wall proper. This thickening disappeared in summer and could be removed with hydrochloric acid from twigs collected in midwinter.

SCHELLENBERG likewise observed that hemicellulose is laid down and utilized as a reserve food in different kinds of tissues of lignified plants, but especially in sieve tubes, wood fibers, and cortex parenchyma. It is removed in the spring from parenchyma cells throughout the plant, from the tips of branches to ends of roots. He points out the fact, moreover, that cells immediately below an inflorescence show conspicuously the disappearance of wall thickenings during flowering. Another interesting observation made by SCHELLENBERG (22) is that in various organs of *Molinia coerulea*, when cell wall thickenings appear after leaf fall (Oct.-Nov.), starch disappears, and when they are redissolved in the spring starch is present once more, thus suggesting a possible connection between starch and hemicellulose. In all instances hemicellulose of this nature could be removed by hydrolysis with hydrochloric and sulphuric acids (3-5 per cent.).

Since then many investigators have noted the hemicellulose layer in cells of various plants and have made attempts to study its chemical nature. The technical horticulturists in particular have determined the presence and the relative amounts of these substances in a number of deciduous trees, especially the apple.

Hemicellulose in apple tissues

Although hemicellulose has been included in chemical analysis of apple wood (12, 9, 7, 13, 2) it has not been recognized as such but has been commonly referred to by the already mentioned terms "polysaccharides," "acid hydrolyzable polysaccharides," etc. This terminology, if justifiable at all, should be used only in cases where starch and other hydrolyzable material has been included in this fraction. Recently, however, the name

"hemicellulose" has come to the forefront. Moreover, it has been recognized that the various sugars and starches are not the only carbohydrates that enter into the metabolism or may serve as storage products, but that hemicellulose should be considered as an important reserve substance in

TABLE I

STARCH (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1925

VARIETAL TISSUES	APRIL 15	MAY 1	MAY 15	JUNE 15	JULY 15	AUGUST 15
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Jonathan						
Leaves	0.40	2.45	3.30	2.40	1.35
New growth . . .	2.15	2.25	3.75	1.15	3.75
Flowers-fruit ...	1.00	2.40	3.60	9.15	14.30	11.20
Paynes Late Keeper						
Leaves	0.75	2.10	4.35	2.80	1.90
New growth .. .	2.00	2.10	4.25	0.40	4.60
Flowers-fruit . .	0.25	1.75	7.45	10.00	11.95	17.70

TABLE II

HEMICELLULOSE (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1925

VARIETAL TISSUES	APRIL 15	MAY 1	MAY 15	JUNE 15	JULY 15	AUGUST 15
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Jonathan						
Leaves	14.50	16.30	13.70	17.80	15.95
New growth . . .	18.20	17.90	16.60	20.40	21.65
Flowers-fruit . .	16.80	13.60	15.20	22.05	18.30	9.55
Paynes Late Keeper						
Leaves	15.55	17.65	14.35	17.25	15.70
New growth . . .	18.90	17.00	18.20	18.75	21.65
Flowers-fruit . .	15.25	14.55	15.70	21.25	22.55	13.95

apple tissues. TOTTINGHAM, *et al.* (29), for instance, have shown that when sugars and starch are removed from apple wood the residue, upon hydrolysis, will produce reducing substances that in most instances represent 2 to 5 times the quantity of both sugars and starch combined. A detailed examination of the products of hydrolysis of this hemicellulose showed that it consists primarily of xylose and glucose with a small amount of galactose. ROBERTS (21) too is of the opinion that in the apple the carbohydrate reserve, in the form of cell wall thickenings, constitutes a greater quantity

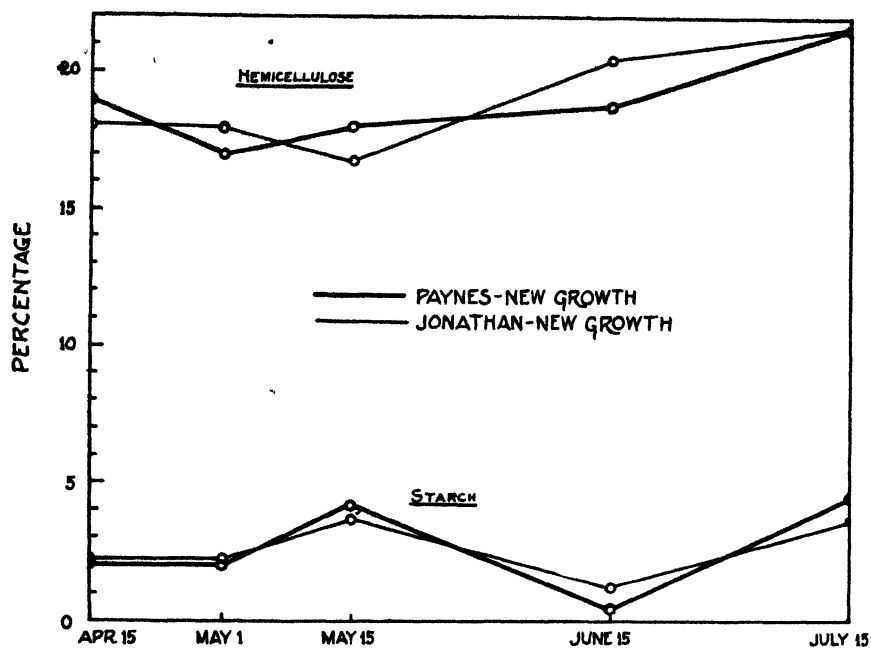


FIG. 1. Hemicellulose and starch concentration, in percentages of dry weight, in new growth of two varieties of bearing apple spurs, 1925.

than that of starch, and that it is laid down primarily in the fall and disappears when growth is resumed. And HARVEY (8) has shown that of the total polysaccharides in apple shoots, by far the largest fraction is made up of pentoses, thus indicating that hemicelluloses are primarily pentosans, as was suggested by SPOEHR (27).

In a recent investigation on the nitrogen and carbohydrate distribution in organs of two varieties of apple spurs the writer (16) has noted once more the conspicuously large amounts of hemicellulose in certain tissues. While during the summer the starch content in the new growth of the fruiting spur may constitute 1 to 4 per cent. of the total dry matter (table I), hemicellulose represents 17 to 22 per cent. (table II and fig. 1). Similar proportions of these two groups of substances were found also in leaves, flowers, and fruits (fig. 2). Hemicellulose appears to be formed in great quantity in the developing fruits, which show a consistent increase to a maximum concentration by midsummer, followed by a rapid decrease later on (fig. 2). The data suggest that hemicellulose is laid down in the flesh or cortex of the fruit as a reserve food in the form of accretions to cell walls which during the later part of the development are hydrolyzed through enzymatic action (see also Amer. Journ. Bot. 10: 310-324. 1923). Thus in

TABLE III
HEMICELLULOSE (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1926

VARIETAL TISSUE	APRIL 26	MAY 1	MAY 6	MAY 11	MAY 17	MAY 22	MAY 27	JUNE 1	JUNE 7	JUNE 12
Jonathan										
Leaves	26.70	27.6	25.2	31.1	28.9	35.5	34.7	30.7	28.10	30.00
New growth	39.6	39.1	21.5	49.7	32.7	29.7	27.1	36.75	28.10	38.45
Flowers-fruit	37.2	29.05	19.1	32.15	32.2	27.5	26.3	26.3	25.80	31.45
King David										
Leaves	27.05	29.3	27.5	33.2	27.6	31.9	29.1	31.65	29.55	30.45
New growth	35.8	35.4	19.8	49.7	35.4	37.93	36.85	36.8	33.20	37.2
Flowers-fruit	28.3	29.35	21.3	29.4	31.2	25.95	27.45	28.3	27.55	33.5

addition to starch, evidently it is a source of increasing sugar content for the maturing fruit.¹

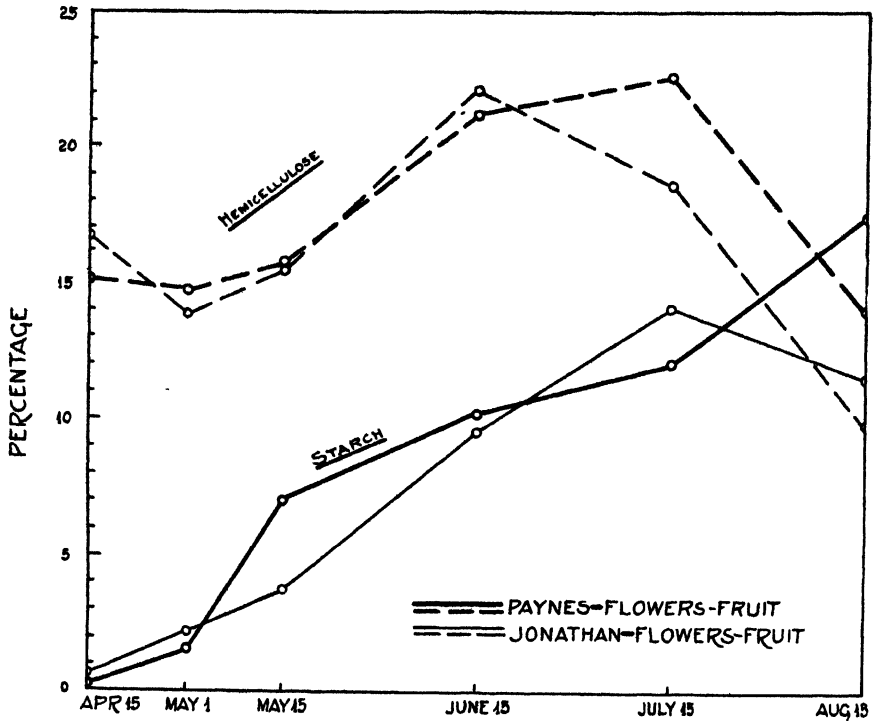


FIG. 2. Hemicellulose and starch concentration, in percentages of dry weight, in reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1925.

When comparable material was analyzed at short 5- to 6-day intervals, other interesting features regarding the behavior of hemicellulose in various organs of the fruiting apple spur were revealed. A very rapid reduction in hemicellulose content seems to have taken place in the new growth and the flowers at the time of anthesis—May 3–9 (table III and fig. 3). And since at the exact time when the concentration of hemicellulose was at a minimum the quantity of total sugars is correspondingly high (table IV and fig. 4), it is more than likely that much of the hemicellulose had been converted into sugars during this period and again recondensed after petal and flower abscission. The starch content, not undergoing an equally rapid fluctuation during this time of intense development (fig. 5), suggests that starch after all is not the most important and most available storage form

¹ It is conceivable that various "pectin" substances may function in the same capacity in the apple fruit and that they may have been included in this determination.

TABLE IV
TOTAL SUGARS (AS DEXTROSE) IN PERCENTAGES OF DRY WEIGHT IN TWO VARIETIES OF BEARING APPLE SPURS, 1926

VARIETAL TISSUE	APRIL 26	MAY 1	MAY 6	MAY 11	MAY 17	MAY 22	MAY 27	JUNE 1	JUNE 7	JUNE 12
<i>Jonathan</i>										
Leaves	4.20	6.30	4.85	3.90	3.00	2.90	4.65	4.50	3.90	5.15
New growth	4.80	3.50	2.33	2.00	1.30	2.60	2.40	2.90	2.80	2.15
Flowers-fruit . . .	2.60	6.20	11.95	2.75	2.95	2.95	4.60	4.80	7.45	7.50
<i>King David</i>										
Leaves	3.45	5.15	7.40	6.10	6.10	4.50	5.35	5.65	4.60	4.90
New growth	4.20	3.45	2.93	1.35	2.93	2.25	2.90	2.35	2.45
Flowers-fruit . . .	2.40	3.30	12.20	4.50	3.05	2.75	4.80	6.15	9.30

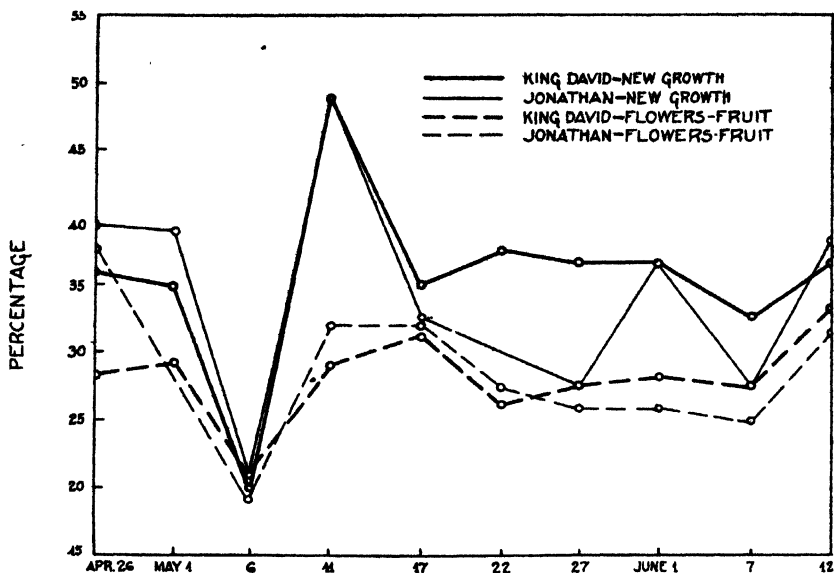


FIG. 3. Graphs showing changes in hemicellulose content during flowering and fruit setting in new growth and reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

of carbohydrates in the apple as has been postulated by some investigators. In fact, the percentage of starch in the new growth of the spur actually increased during flowering (May 6, fig. 5)—the period of greatest reduction in the hemicellulose content. Hence there is a possibility that, concomitant with the rapid increase in sugar content, starch may have been synthesized from the products of hydrolysis of hemicellulose. This seems to point to a starch-hemicellulose equilibrium and thereby support is given to SCHELLENBERG's observations (22).

These studies emphasize once more the importance of hemicellulose as a reserve carbohydrate in woody plants. Hence this group of substances most certainly merits further detailed study with special emphasis on the chemical constitution and metabolic rôle of each constituent.

Chemistry of hemicelluloses

Although one is forced to agree with SPOEHR (*loc. cit.*) that though much literature has accumulated on the subject of the colloidal polysaccharides (including hemicelluloses), the "opinions are widely divergent and the subject in general is a most uncoordinated one," still, lately a number

of contributions have appeared on the chemistry of hemicelluloses which seem to merit our attention.

In the make up of plant cell walls three major substances commonly accompany cellulose. They are: lignin, hemicellulose, and pectin. None of them, however, as yet has been isolated in chemically pure form. From all appearance they seem to be closely related to each other, this being particularly true of pectin and hemicellulose. Consequently any progress made in the chemistry of one of these groups of substances may throw light upon the others.

In addition to cellulose, woody tissues usually contain lignin and hemicellulose while non-lignified portions of plants show only small amounts of hemicellulose, but relatively large aggregations of pectin. During lignification pectin seems to disappear and is replaced by hemicellulose and lignin. CANDLIN and SCHRYVER (3) even go so far as to advance the idea that hemicellulose in lignified cells is derived from pectin and that there is a possibility, though remote as yet, that certain products obtained from pectin may be related to lignin. EHRLICH (6) likewise says that hemicellulose is closely related to pectin and that lignin is probably formed from pectin by oxidation processes. Thus it is at least suggestive that these three

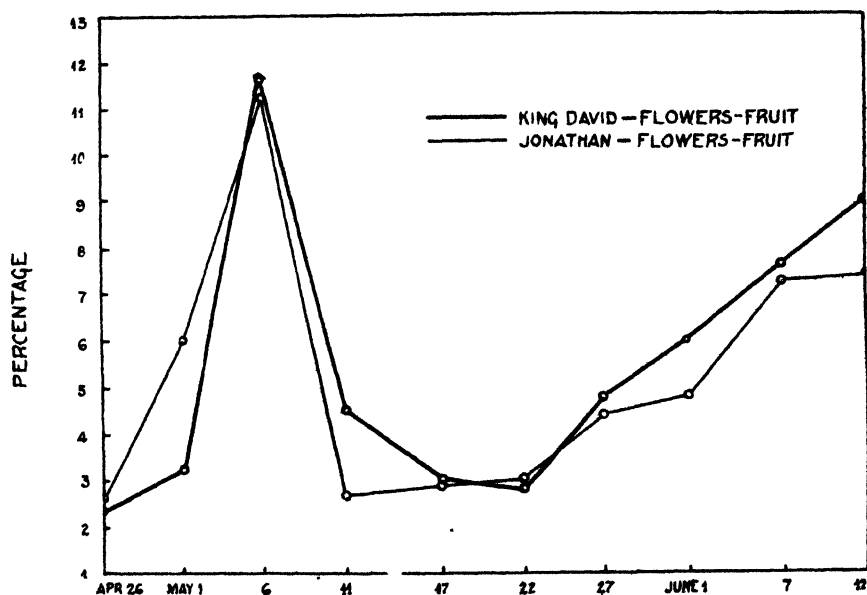


FIG. 4. Graphs showing changes in total sugar content during flowering and fruit setting in reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

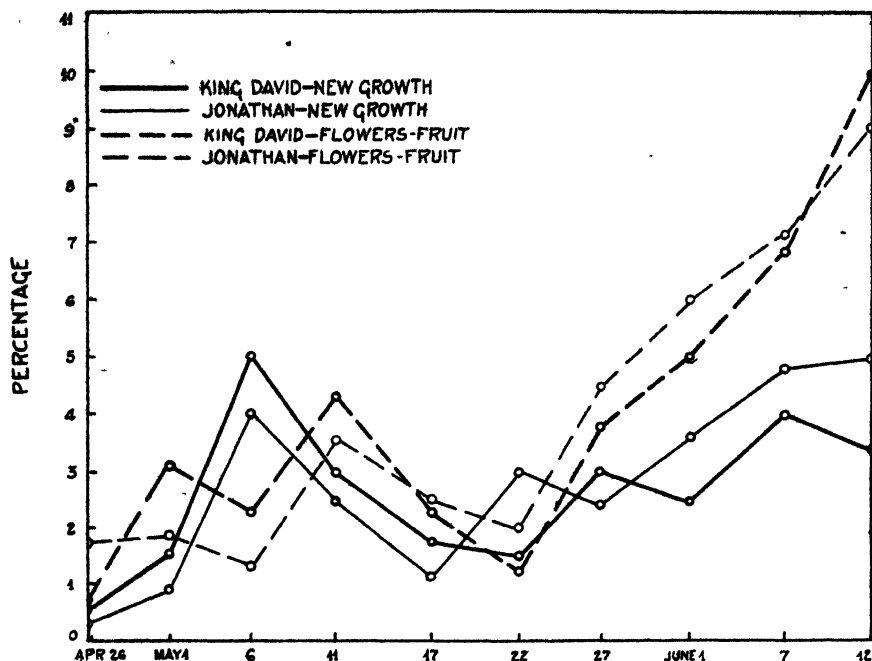


FIG. 5. Graphs showing changes in starch content during flowering and fruit setting in new growth and reproductive organs (flowers-fruit) of two varieties of bearing apple spurs, 1926. Percentages on dry weight basis.

interesting compounds of the plant cell wall may be generically connected. Furthermore, recent studies by SCHRYVER and coworkers (4, 25) and by LING and NANJI (14) seem to indicate that a substance of the nature of hemicellulose ("amylo-hemicellulose") is present in various starches (sago, maize, wheat, rice, tapioca, potato). Unlike the main constituents of starch, this hemicellulose is not digested by diastase. When hydrolyzed by acids it yields largely glucose. Some of the authors (14) believe that "amylo-hemicellulose" is present not only in starch but also as a principal constituent of cell walls of many plant organs, especially during early stages of development. As it colors blue with iodine it may be taken for starch. THAYSEN and BUNKER (28) think that hemicellulose probably is more common in plant tissues than cellulose, but is less thoroughly investigated.

A number of reagents have been used for the extraction of hemicellulose but a dilute solution of caustic soda (2-4 per cent.) (18) and hot water, with or without autoclaving (10), seem to have been preferred. While for the extraction of pectin a weak solution (0.5 per cent.) of ammonium oxalate (*loc. cit.* 3) is usually employed, although hot and cold water have also been used extensively. Therefore, when literature on this phase of the

subject is reviewed one can not help gaining the impression that, with some plant material at least, a water extract may not represent pure hemicellulose or pectin but a mixture of the two, and that a solution of alkali (4 per cent.) most likely decomposes hemicellulose. Evidently a proper medium for the separation of hemicellulose from other cell wall constituents is yet to be found.

When a detailed chemical investigation is undertaken, the extracted hemicellulose, with or without further purification, is usually subjected to an acid hydrolysis. The end products of such a treatment have been popularly considered as consisting of a mixture of several monosaccharides—hexoses and pentoses. The following sugars have been often identified as the products of hydrolysis of various hemicelluloses: glucose, galactose, mannose, arabinose, xylose and others. While some workers are of the opinion that mannans and galactans are the most common forms of reserve celluloses, most hemicelluloses evidently are made up largely of pentoses and hence may be considered as pentosans. PRINGSHEIM (19), however, has isolated from certain hemicelluloses a heretofore unknown trisaccharide and a dimannan.

Lately quite convincing proof has been secured that substances of the type of galacturonic and glycuronic acids and their polymeric anhydrides, the poly-galacturonic and -glycuronic acids appear to be the main building stones from which plant cell membranes (hemicelluloses), and pectins as well, are constructed (6, 18). This particular group of polysaccharides has the characteristics of both carbohydrates and acids. And since these "uronic" acids either make up the nucleus (15, 1) or constitute a large fraction of the pectin compounds (24), this may be considered as another link in the evidence of the close relationship between pectins and hemicelluloses. Thus both of these cell wall constituents may be called "poly-uronides."

Pectins probably contain more uronic acids than hemicelluloses, but lignin contains none (3). It may be of further interest to note that according to CANDLIN and SCHRYVER, pectin, when treated with weak alkali yields among other products hemicelluloses, which still contain uronic acids and resemble in some respects hemicelluloses isolated directly from timber. It is quite evident then that a rather interesting field of investigation has been opened by recent studies of the various complex carbohydrates which are not merely aggregates of the plant cell wall but also serve as reserve substances.

In spite of the somewhat involved chemistry and the complex "uronic" acids in particular, the distribution and rôle of hemicellulose should be of special interest to plant physiologists. In woody plants these

substances are usually present in very large amounts and frequently serve as reserve carbohydrates. Moreover, the probable relationship between starch, pectin, hemicellulose and lignin is very interesting indeed. As pentosans, hemicelluloses seem to be very effective in making plants resistant to drought and to cold. This special field of investigation has been only tentatively explored. Finally the hemicelluloses seem to be of consequence in relation to the carbohydrate-nitrogen ratio in plants. Frequently the acid hydrolyzable polysaccharides (hemicelluloses, or hemicelluloses and starch) constitute the preponderant amount of all the available and extractable carbohydrates and consequently may successfully represent them in the ratio (17). Moreover, HICKS (11) has shown rather clearly that the ratio of total elemental carbon to total elemental nitrogen, the true C/N ratio, may be just as significant as that of carbohydrates to nitrogen. One should remember that in many instances the hemicellulose represents a very large part of the carbon and the carbohydrates.

UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI

LITERATURE CITED²

1. AHMANN, C. F. and HOOKER, H. D. The estimation of pectin and a study of the constitution of pectin. Missouri Agr. Exp. Sta. Res. Bull. 77. 1925.
2. AUCHTER, E. C., *et al.* The effect of shade on the growth, fruit bud formation, and chemical composition of apple trees. Proc. Amer. Soc. Hort. Sci. 1926: 368-382.
3. CANDLIN, E. J. and SCHRYVER, S. B. Investigations of the cell-wall substances of plants, with special reference to the chemical changes taking place during lignification. Proc. Roy. Soc. (London) B. 103: 365-376. 1928.
4. CLAYSON, D. H. F. and SCHRYVER, S. B. The hemicelluloses. I. The hemicellulose of wheat flour. Biochem. Jour. 17: 493-496. 1923.
5. DU SABLON, LECLERC. Recherches physiologiques sur les matières de réserves des arbres. Rev. Gen. Bot. 16: 341-368, 386-401. 1904.
6. EHRLICH, F. Neue Untersuchungen über Pektinstoffe. Zeitschr. Ang. Chem. 40: 1305-1313. 1927.
7. HARLEY, C. P. Normal variations in the chemical composition of fruit spurs and the relation of composition to fruit bud formation. Proc. Amer. Soc. Hort. Sci. 1925: 134-146.

² Acknowledgment is due to Prof. W. E. PYKE, of the Department of Chemistry, Colorado Agricultural College, for suggesting some of the references.

8. HARVEY, E. M. A study of growth in summer shoots of the apple with special consideration of the rôle of carbohydrates and nitrogen. Oregon Agr. Exp. Sta. Bull. 200. 1923.
9. ———, and MURNEEK, A. E. The relation of carbohydrates and nitrogen to the behavior of apple spurs. Oregon Agr. Exp. Sta. Bull. 176. 1921.
10. HENDERSON, S. T. The pectin and hemicelluloses of the flax plant. Jour. Chem. Soc. (London) 130: 2117–2125. 1928.
11. HICKS, P. A. The carbon-nitrogen ratio in the wheat plant. New Phyt. 27: 1–46. 1928. *Ibid.* 27: 108–116. 1928.
12. HOOKER, H. D. Seasonal changes in the chemical composition of apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 40. 1920.
13. KRAYBILL, H. R., *et al.* Some chemical constituents of fruit spurs associated with blossom bud formation in the Baldwin apple. New Hampshire Agr. Exp. Sta. Tech. Bull. 29. 1925.
14. LING, A. R. and NANJI, D. R. Studies on starch. Part IV. The nature of the amylo-hemicellulose constituent of certain starches. Jour. Chem. Soc. (London) 127: 652–656. 1925.
15. MCKINNIS, R. B. An investigation of the hypothetical combined pentose and the so-called free pentose with inferences on the composition of pectin. Jour. Amer. Chem. Soc. 50: 1911–1915. 1928.
16. MURNEEK, A. E. Nitrogen and carbohydrate distribution in organs of bearing apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 119. 1928.
17. ———. Effects of pruning on the carbonhydrate-nitrogen ratio in the tomato. Proc. Amer. Soc. Hort. Sci. 1927: 180–184.
18. O'DWYER, M. H. The hemicelluloses. III. The hemicelluloses of American white oak. Biochem. Jour. 17: 501–509. 1923. *Ibid.* 20: 656–664. 1926; 22: 381–390. 1928.
19. PRINGSHEIM, H. Über den fermentativen Abbau der Hemicellulosen. I. Ein Trisaccharid als Zwischenproduct der Hydrolyse eines Mannans. Zeitschr. Physiol. Chem. 80: 376–382. 1912. *Ibid.* 123: 205–212. 1922.
20. REISS, R. Über die Natur der Reservecellulose und über ihre Auflösungsweise bei der Keimung der Samen. Landw. Jahrb. 18: 711–765. 1889.
21. ROBERTS, R. H. Apple physiology. Wisconsin Agr. Exp. Sta. Res. Bull. 68. 1926.
22. SCHELLENBERG, H. C. Über die Bestockungsverhältnisse von *Molinia coerulea* Mönch. Ber. Schweiz. bot. Ges. 1897.
23. ———. Über Hemicellulose als Reservestoffe bei unseren Waldbäumen. Ber. deutsch. bot. Ges. 23: 36–48. 1905.

24. SCHRYVER, S. B. and NORRIS, F. W. The pectic substances of plants. III. The nature of pectinogen and its relation to pectic acid. *Biochem. Jour.* **19**: 676-693. 1925.
25. ————— and THOMAS, E. M. The hemicelluloses. II. The hemicellulose contents of starches. *Biochem. Jour.* **17**: 497-500. 1923.
26. SCHULZE, E. Zur Kenntniss der chemischen Zusammensetzung der pflanzlichen Zellmembranen. *Ber. deutsch. chem. Ges.* **24**: 2277-2287. 1891. *Idem.* *Zeitschr. Physiol. Chem.* **16**: 387-438. 1892.
27. SPOEHR, H. A. The carbohydrate economy of cacti. *Carnegie Inst. Washington. Publ. no. 287.* 1919.
28. THAYSEN, A. C., and BUNKER, H. J. The microbiology of cellulose, hemicellulose, pectin, and gums. *Oxford Univ. Press.* 1927.
29. TOTTINGHAM, W. E., *et al.* Hemicellulose in apple wood. *Jour. Biol. Chem.* **45**: 407-414. 1921.

IDENTIFICATION OF CERTAIN SPECIES OF CITRUS BY COLORIMETRIC TESTS¹

F. F. HALMA AND A. R. C. HAAS

Citrus varieties are propagated commercially by budding. In California four species are used for rootstocks and in the order of their importance, they are as follows: Sweet orange (*Citrus sinensis* Osbeck), sour orange (*Citrus aurantium* L.), grapefruit (*Citrus maxima* Merrill), and rough lemon (*Citrus limonia*). *Poncirus trifoliata* Raf., used formerly to a limited extent, is being discarded.

At the present time considerable thought is being given to the selection of stocks which are congenial with the scion variety. Owing to the fact that formerly very little attention was paid to this phase of citriculture, we now find a considerable mixture of stocks in older plantings. Such old orchards would offer excellent opportunity to study the interrelation of stock and scion were it possible to identify definitely the stock in question. Up to the present time there have been no generally applicable methods by which this could be done. Occasionally one finds a sprout growing from below the bud union, or from a cut root, from which it is possible to make a diagnosis. The type of bud union sometimes suggests the stock variety, but it is always difficult to distinguish between sweet orange and sour orange stocks, especially with old trees.

For the past two years considerable effort has been made to devise a method which would at least make it possible to separate sweet orange from sour orange stocks with certainty. It is obvious that any adequate method must be accurate and fairly rapid, and must require very little material. It may be mentioned that a separation on the basis of anatomical differences was found to be unsatisfactory. Water extracts of powdered bark when tested with different pH indicators (within, as well as outside of the range of citrus-bark acidity), gave inconsistent results.

Recently HENDRICKSEN (2) published a method which is based on the fact that all Citrus roots contain varying amounts of glucosides and that these glucosides contain phenols. By treating an aqueous extract of ground fresh roots with ferric chloride, he observed different shades of color with the sour orange, "wild" grapefruit, and Rough lemon. Sweet orange gave inconsistent results. As a matter of fact HENDRICKSEN considers this method reliable only for Rough lemon, and hence it is of very little value for California conditions. There is the additional disadvantage of having to work with fresh material.

¹ Paper no. 206, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

This paper reports a series of successful tests which were carried on with powdered bark. Of the numerous reagents used, some of which will be mentioned later, the ALMÉN test (1) for carbolic or salicylic acid gave the most consistent results. This reagent is prepared as follows: Saturate fuming nitric acid with mercury and dilute the solution with double its volume of water. The degree of sensitiveness is given as 1:400,000. This reagent is practically the same as Millon's reagent for albumins and phenols (1).

The procedure which has given excellent results is as follows: Enough bark is peeled from the trunk below the bud union to make at least one gram when dried at about 70° C. Adhering soil or foreign matter, of course, must be removed from the bark before taking the sample. The dried bark is ground fine enough in a mortar to pass through a 40-mesh sieve. One gram of this powder is placed in a beaker and 20 cc. of distilled water are added. In a few minutes the solution is ready for filtration. The contents of the beaker are transferred to filter paper and the residue is washed with two successive 20-cc. portions of distilled water. The one gram of powder is therefore leached with 60 cc. (altogether) of water.

Ten cubic centimeters of the filtrate are measured into a test tube and 8 drops of the ALMÉN reagent are added. A heavy yellowish precipitate forms immediately. The contents of the tube are then boiled a few minutes in a water-bath or in an open flame. Within a short time the heavy precipitate assumes varying shades of color and the solution itself becomes colored. Even at this stage it is often possible to distinguish sweet orange by its deep pink color, but it is best to let the solution cool and the precipitate settle before a critical examination is made. Frequent shaking seems to accentuate the color of the solution upon settling.

With sweet orange the solution assumes a characteristic pink color which persists for several weeks. The grapefruit solution shows a much lighter pink, and the color fades much sooner than that developed by sweet orange. Sour orange usually develops a light pink color and quite often it is difficult to distinguish it from that given by grapefruit. It was found, however, that this difficulty can largely be overcome by frequently shaking the solutions and allowing them to stand several hours. The Rough lemon gives the lightest color of all and with a little experience no special difficulties are encountered in its determination.

A modification of this method which shows greater color differences in a much shorter period, is as follows: Ten cubic centimeters of the filtrate are made alkaline with a few drops of potassium hydroxide prior to the addition of 8 drops of a saturated solution of copper sulphate. This results in the formation of a heavy precipitate. Eight drops of the mercury reagent are then added and the contents of the tube are boiled. The color of the sweet orange solution becomes pink as in the other method but the sour

orange solution assumes a brown color which can be at once distinguished from that of sweet orange. The color of the grapefruit solution becomes a light pink and that of the Rough lemon almost colorless. The sweet orange solutions show a very narrow range of pink while the sour orange and grapefruit solutions fluctuate in some cases over a comparatively wide range within their color group.

If added confidence is needed the remaining portion of the liquid may be used in the following supplementary tests. These methods will be found helpful, although they are not as reliable as those described above; they separate the species only roughly in some cases, but at other times quite definitely. Only 5 cc. of solution are needed for each of the additional tests.

Ammonium molybdate is the reagent ordinarily used for phosphorus determinations and is prepared as follows: Dissolve 100 grams of molybdic acid in a mixture of 144 cc. of ammonium hydroxide and 271 cc. of water. Pour slowly into a cool mixture of 489 cc. of nitric acid and 1148 cc. of water. Ten drops of this reagent added to the liquid and boiled, will give rather consistently a deep blue color with Rough lemon and a much lighter blue or bluish gray with grapefruit. Sweet and sour orange give varying shades of green.

The addition of 10 drops of a saturated solution of copper sulphate roughly separates sweet and sour orange as a single group from grapefruit and Rough lemon as another group. The color differences can be somewhat intensified by the addition of 2 drops of aniline violet gentian (0.01 gram dissolved in 75 cc. of water).

Two drops of titanous chlorid (20 per cent. solution) gives a very light pink color with Rough lemon, a warm gray with grapefruit, and a greenish color with both sweet and sour orange.

Two drops of a saturated solution of ferric chlorid often give a brownish fluorescent turbidity with sweet orange, a dark clear brown color with sour orange, and a clear straw color with Rough lemon and grapefruit (Rough lemon solution generally gives a lighter color than grapefruit).

Many other reagents were tested some of which gave promising results, but the consideration of which would add very little to the previous discussion. The diversity of reagents used precludes any definite statement as regards the nature of the principle involved. It may be mentioned that twelve different phenol reagents gave negative results. Even the mercury reagent gave results somewhat different from the characteristic reactions obtained with phenol or protein.

In a preliminary trial of these methods of identification, bark samples from trees of definitely known variety were examined. More than 100 samples each of sweet orange and sour orange bark, more than 50 of grapefruit

bark, and about 20 of Rough lemon bark were tested without a single discrepancy.

A considerable number of unknown bark samples representing the four kinds of rootstocks have also been successfully identified. A set of 37 samples of bark (11 each of sour, sweet, and grapefruit, and 4 of Rough lemon) was obtained from the citrus breeding orchard of the Citrus Experiment Station and a set of 100 samples representing both sweet and sour orange rootstocks. In each case we determined numbered samples, to whose identity we had no clue aside from the chemical tests, and the list of determinations was given to a cooperator who had retained the only key to the sample numbers. All samples were correctly determined. The odds against the accidental occurrence of this result are obviously enormously large.

The results of these tests are not influenced by the age of the tree, by the time of the year at which the samples were taken, nor by the scion variety; in fact, the color obtained within a certain group is the same regardless of whether it serves as scion or as stock.

With the exception mentioned below, the results with the mercury test have been uniform within the commonly accepted limits of the species here considered. The sweet orange barks tested include, in addition to rootstocks (probably representing the "Mission seedling" type) the varieties Ruby, Valencia, and Washington Navel, while the known grapefruit varieties include Marsh, Duncan, Triumph, and the decidedly different Imperial. The sour orange barks tested undoubtedly represent a mixed lot of unknown origin. On the other hand, the standard California lemon varieties (Lisbon and Eureka) give a characteristic rusty red color which is quite different from that obtained with Rough lemon, although all these lemons are commonly included in *Citrus limonia*. These results suggest the possibility that these colorimetric differences may be useful in citrus classification.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. COHN, A. I. Tests and reagents. John Wiley and Sons, New York. 1909.
2. HENDRICKSEN, H. C. Identification of citrus budding stock: Porto Rican method for testing root system. *Florida Grower* **36**: 14-15. 1928.

THE EFFECT OF POTASSIUM, NITROGEN AND PHOSPHORUS FERTILIZING UPON THE CHLOROPLAST PIGMENTS, UPON THE MINERAL CONTENT OF THE LEAVES, AND UPON PRODUCTION IN CROP PLANTS¹

F. M. SCHERTZ

Introduction

In a former paper (8) by the writer, the effect of nitrogen on the chloroplast pigments has been especially indicated. The effect of nitrogen upon the growth of plants is perhaps greater than that of any of the other elements.

VILLE (14) was perhaps the first to make any quantitative experiments on the effect of the various fertilizers upon the pigment content of plants. He has shown that nitrogen plays a rôle more important than that of any of the other elements tested. If the dose of nitrogen was increased or diminished the color increased or diminished also.

URBAN (13) found evidence of a correlation between the color of the leaves of beets and the nitrogen content, the darker leaves containing the most nitrogen and the lighter the least. Also, he apparently found a relation between the highest potassium content of the leaves and the greatest sugar content of the roots. In beets the dark colored leaves contained more potassium and less sodium than did the light colored leaves. In the leaves of ripening beets the potassium rapidly increases while the sodium decreases.

A great difference in the chlorophyll content of alpine and of lowland plants was observed by HENRICI (2). She represented the amount of chlorophyll present in alpine plants as 100 per cent. and found that lowland plants contained 230 per cent. and ravine plants 350 per cent. She makes no reference to the soil in which these grew but it is highly probable that the soil was a very important factor in the amount of chlorophyll which was found in the plants studied.

Perhaps the first real experiments on the effect of chemical fertilizers upon chlorophyll were conducted by WLODEK (16) in 1920. Green leaves of potato plants and sugar beets were studied. These were grown in soils to which various fertilizers were added: (1) without fertilizer; (2) with phosphorus, potash and nitrogen; (3) with phosphorus and nitrogen but no potash; (4) with phosphorus, nitrogen and magnesium but no potash; (5) with potash and nitrogen but no phosphorus; and (6) with potash and phosphorus but no nitrogen. After a certain period of development of the

¹ Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

plants it was concluded that the relation of the chlorophyll pigments varies during the course of 24 hours; β increases during the day and α during the night. The lack of potash resulted in an absolute and relative diminution of β and an increase in α as well as a reduction in the daily variation of the two components. Lack of phosphorus also reduces the daily variation of the chlorophyll components and narrows the absorption bands. Nitrogen tends to reduce α and to augment β . The action of calcium and magnesium were not definitely established.

In another paper WLODEK (17) has reported that with a lack of potash the chlorophyll coefficient diminished at a certain phase of development of the plants. This is shown by an increase in the width of the absorption band of chlorophyll α and a decrease in that of chlorophyll β , as compared with plants grown under normal conditions. A lack of nitrogen in the soil increases the chlorophyll coefficient. A relation seems to exist between the width of the first absorption band and the nitrogen content in fresh bean leaves, and in the straw and harvest of oats and barley. The nitrogen content increases with the width of the absorption band. When potash is lacking the chlorophyll coefficient does not change under the influence of light and darkness. In leaves which show an abnormal chlorophyll coefficient the amount of vegetative material which is produced is less than in those which possess the normal chlorophyll coefficient.

In studying the bush characteristics of potato plants, SCHAEFER (7) has placed the leaves in four groups as to color. The classes were, bright green, green, green to dark green, and dark green. He thought that possibly nitrogen affected the color.

WEISSMANN (15) noticed that potash influenced the development of plants and their morphological and anatomical structures. He found also that deficient potash affects the coloring of the leaf, stem and the grain. Deficient phosphorus and nitrogen more strikingly affect the early stages of growth than does a deficiency in potash.

In making chemical analyses of leaves, CHIBNALL (1) learned that there was a nitrogen withdrawal from the leaves at night. This fact may aid us in understanding the nitrogen metabolism of the leaf.

Studies by MAIWALD (3) lead to the conclusion that different applications of potassium produced a great difference in the color of the potato leaves of the same species. The leaves varied in color from a dark green to a yellow green, the yellow green ones resulting when much potash was applied. When based on green weight, deviations of the chlorophyll content were found to be 70 per cent. above and below normal. The amount of the chlorophyll present was the sole cause of the striking color difference in the leaves. By comparing the assimilating power of the plant with the

chlorophyll content, the author concludes that the abnormally low chlorophyll content is the beginning of chlorosis.

Potassium sulphate was found to reduce the chlorophyll content of sound green potato leaves, while kainit and potassium chloride were found by REMY and LIESEGANG (5) to greatly reduce the amount of chlorophyll present. Healthy leaves of pronounced potash hungry plants always contained more chlorophyll per unit weight than potash satisfied plants; the same is also true of sugar beets. Leaves of potatoes which had been treated with large amounts of potash salts contained less chlorophyll than did leaves from potatoes receiving less potash salts. Plants treated with potash have a larger growth than plants not treated, consequently in spite of the fact that the content of chlorophyll per gram of leaf is less, each plant actually has more chlorophyll in it. Leaves of plants fertilized with potash possess the power to assimilate for a greater length of time than do those not fertilized.

In studying the effect of nitrate applications upon the hydrocyanic acid content of sorghum, PINCKNEY (4), found that nitrogen affected the size and the color of the plants to such an extent that sorghum may be used as an indicator of the amount of available nitrogen in the soils. Plants grown in soils containing readily available nitrogen grew rapidly and were much greener in color than plants grown in soils which did not contain available nitrogen.

Sir JOHN RUSSELL (6) has discussed the effects of nitrogen, phosphorus and potash upon plant growth. Nitrogenous fertilizers increase the rate of leaf growth and so produce larger leaves and stems. Also, they induce a greater formation of green coloring matter, giving darker green crops. Phosphates on the other hand greatly increase the root growth.

Methods and materials

The methods used for extracting and separating the pigments were those described in a previous paper (11) by the writer. The amount of chlorophyll present was measured by the colorimetric method outlined in the paper on the quantitative determination of chlorophyll (12). The carotinoids were estimated by means of the colorimetric methods (9, 10), devised by the writer. The spectrophotometric methods were not used here for they had not been developed at the time the work described in this paper had been completed.

The materials used were collected from various field fertilizer experiments of this department. The cotton leaves were from the field experiments of J. J. SKINNER and the potato leaves from those of B. E. BROWN. An attempt was always made to collect representative samples of each fertilizer plot. The samples were forwarded in waxed paper, to

prevent drying of the sample, to Washington, D. C., and the analysis was completed in this laboratory.

The numbers 1, 16, 21, 7, 8, 9, and 10 in the tables refer to that portion of the P. N. K. fertilizer triangle, in use in this department, from which the samples were taken. The composition of these fertilizers is shown in the following table I.

TABLE I
COMPOSITION OF FERTILIZERS USED IN THESE STUDIES

TREATMENT NUMBER	FORMULA		
	NH ₃	P ₂ O ₅	K ₂ O
1	0	20	0
16	0	0	20
21	20	0	0
7	0	8	12
8	4	8	8
9	8	8	4
10	12	8	0

The elements N, P, K, and S, were determined by the following methods, which need not be described in detail here. Total nitrogen was determined by the Kjeldahl method as modified by GUNNING and ARNOLD while total phosphorus was estimated by the NEUMANN-PEMBERTON method. Sulphur was estimated by the fusion method, and potash was estimated by the Assoc. of Offic. Agr. Chemists' method substantially as given in the year 1919.

Results

The pigment results were all obtained by a direct comparison with Lovibond slides, the readings from which were later interpreted in terms of grams of pigment. Part of the leaves were dried and part of them were extracted in the fresh condition. For the chemical analysis the leaves were all dried according to regular laboratory procedure. The dried leaves for extraction were then ground to a fine powder with sand in a ball mill before extraction.

In general, a survey of tables II and III shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in potash or nitrogen. Leaves from plots high in nitrogen contained more of the chloroplast pigments than did the leaves from plots from other parts of the triangle.

Plants fertilized with a mixture high in nitrogen always produced plants whose leaves were also high in nitrogen. The plants which were fertilized

TABLE II
ANALYSIS OF DRIED COTTON LEAVES¹ COLLECTED AT NEWBERN, NORTH CAROLINA,
JULY 23, 1919

NUMBER OF PLOT	1	16	21	7	8	9	10
Chlorophyll ($\alpha + \beta$)	21.0	26.8	29.4	28.6	22.4	31.8	31.8
N	4.97	5.22	6.03	5.14	5.09	5.31	5.50
P	0.72	0.52	0.50	0.60	0.61	0.49	0.50
K	1.34	1.88	2.09	1.37	1.86	1.99	2.33
S	1.35	1.32	1.18	1.43	1.31	1.44	1.23
Pounds of cotton per acre	1424.0	1080.0	2176.0	1434.0	1802.0	2262.0	2528.0

COLLECTED AT FLORENCE, SOUTH CAROLINA, JULY 26, 1919

Chlorophyll ($\alpha + \beta$)	21.0	25.0	26.0	17.6	27.6	25.0	25.0
N	2.98	3.30	5.25	3.13	3.48	4.60	4.91
P	1.03	0.52	0.32	0.37	0.34	0.48	0.51
K	0.89	1.79	1.15	1.68	1.54	1.25	1.17
S	1.52	0.96	1.05	1.49	1.53	1.17	1.05
Pounds of cotton per acre	644.0	770.0	1662.0	888.0	1426.0	1948.0	1850.0

PEE DEE EXPERIMENTAL STATION

COLLECTED AT FLORENCE, SOUTH CAROLINA, AUGUST 29, 1919

Chlorophyll ($\alpha + \beta$)	22.4	23.2	31.8	20.2	18.4	29.4	31.0
N	2.71	3.12	4.62	2.50	2.46	2.31	3.82
P	0.61	0.48	0.36	0.50	0.37	0.31	0.32
K	1.70	2.70	1.14	1.86	1.44	0.98	1.03
S	1.18	1.09	0.74	0.95	0.64	0.62	0.84

COLLECTED SEPTEMBER 21, 1919

Chlorophyll ($\alpha + \beta$)	24.2	15.8	22.0	16.6	23.2	17.6	26.8
Pounds of cotton per acre	1780.0	1640.0	2280.0	1372.0	1676.0	2150.0	2130.0

¹ The results reported for the chloroplast pigments are given in milligrams per 10 grams of fresh leaves. The chemical analyses are reported in grams of N, P, K or S per 100 grams of dry leaves, i.e., in per cent. Data obtained for the carotinoids are not given, for drying the leaves renders these data worthless.

with a mixture high in phosphorus always produced plants whose leaves were high in phosphorus. Plants which were fertilized with a mixture high in potash always produced cotton plants with leaves high in potash. Generally the leaves of plants high in nitrogen contained the least sulphur while those high in phosphorus contained the most sulphur.

TABLE III
ANALYSIS OF FRESH COTTON LEAVES, PEE DEE EXPERIMENT STATION. COLLECTED
AT FLORENCE, SOUTH CAROLINA, JUNE 25, 1920

NUMBER OF PLOT	1	16	21	7	8	9	10
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Carotin	0.66	0.90	0.86	0.57	0.63	0.76	0.76
Xanthophyll	1.20	1.70	2.00	2.00	1.50	1.50	1.30
Total carotinoids.....	1.86	2.60	2.86	2.57	2.13	2.26	2.06
Chlorophyll ($\alpha + \beta$)	19.0	26.8	27.4	22.0	23.4	23.0	23.4

COLLECTED SEPTEMBER 29, 1920

Carotin	0.70	0.78	0.90	0.78	0.86	0.86	0.75
Xanthophyll	1.60	1.03	1.16	1.00	1.30	1.50	1.16
Total carotinoids	2.30	1.81	2.06	1.78	2.16	2.36	1.91
Chlorophyll ($\alpha + \beta$)	16.6	14.8	16.6	13.6	14.8	14.8	16.00
Pounds of cotton per acre	1520.0	1040.0	938.0	1600.0	1608.0	2004.0	1620.0

In general, it may be concluded that plots high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre, although this is not true for all types of soil. The phosphorus plots produced yields which were between those for potash and nitrogen.

A summary of the results from the potato plots (table IV) shows that the plots fertilized with a mixture high in phosphorus produced leaves which contained less chlorophyll than did leaves from plots high in nitrogen. Leaves from plots high in potash evidently contained less chloroplast pigments than did leaves from the other plots.

Potato plants fertilized with a mixture high in nitrogen produced plants whose leaves were high in nitrogen. Plants which were fertilized with a mixture high in potash produced plants whose leaves contained the least potash. Plants fertilized with a mixture high in phosphorus apparently produced leaves which were also high in phosphorus. The amount of sulphur in the leaves could not be correlated with the amount of nitrogen, phosphorus, or potash present in the leaves or that added to the soil as fertilizer.

In table V results are given for a fertilizer experiment at Arlington Farm. In this case, the results are not at all in conformity with what might have been expected and the chloroplast pigments cannot be correlated in any way with the amount of fertilizer added. However, the table shows that as the plants matured the total carotinoids increased from 2.5 to 3.27 mg. as also did the total chlorophyll from 12.8 to 15.3 mg. in each

TABLE IV
ANALYSIS OF POTATO LEAVES, DRIED BEFORE EXTRACTION
IRISH COBBLER VARIETY
COLLECTED MAY 29, 1919, NORFOLK, VIRGINIA

NUMBER OF PLOT	1	16	21	7	8	9	10
	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Chlorophyll ($\alpha + \beta$)	15.5	15.9	18.0

COLLECTED JUNE 17, 1919, NORFOLK, VIRGINIA

Chlorophyll ($\alpha + \beta$)	16.8	15.2	21.1	18.5	16.4	15.9	19.4
Yield (bushels per acre)	81.0	73.0	97.0	75.0	178.0	193.0	167.0

GREEN MOUNTAIN VARIETY

COLLECTED JULY 15, 1919, RIVERHEAD, LONG ISLAND

Chlorophyll ($\alpha + \beta$)	26.0	19.2	32.8	21.0	28.6	35.2	31.8
Yield (bushels per acre)	176.0	191.0	91.0	166.0	192.0	181.0	187.0

NORCROSS VARIETY

COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE

Chlorophyll ($\alpha + \beta$)	12.6	32.8	35.2	39.6	35.2	34.6	38.6
N	4.87	4.60	6.02	4.69	5.15	5.44	6.23
P	0.35	0.27	0.36	0.32	0.21	0.31	0.39
K	2.63	2.08	2.37	2.06	2.09	1.81
S	0.35	0.34	0.36	0.33	0.33	0.41	0.39
No yields available							

IRISH COBBLER VARIETY

COLLECTED AUGUST 4, 1919, PRESQUE ISLE, MAINE

Chlorophyll ($\alpha + \beta$)	19.2	14.0	18.4	14.0	26.0	14.8	20.2
N	4.49	5.15	6.00	4.71	5.10	5.91	5.41
P	0.37	0.30	0.26	0.29	0.29	0.39	0.34
K	2.34	2.25	2.53	3.83	3.75	3.10	1.45
S	0.55	0.72	0.63	0.45	0.71	0.40	0.42
Yield (bushels per acre)	165.0	192.0	153.0	219.0	328.0	336.0	288.0

ten gram sample of the fresh leaves. The samples of leaves which were dried show that the amount of carotin and xanthophyll lost in drying was quite large and consequently the results are unreliable, while the loss of

TABLE V
CHLOROPLAST ANALYSIS OF FRESH POTATO LEAVES WHICH WERE COLLECTED AT DIFFERENT STAGES OF GROWTH
GROWN AT ARLINGTON FARM, VIRGINIA

PLOT NUMBER	DATE OF COLLECTION	1	16	21	7	8	9	10	CHECK	CHECK DRIED
CAROTIN	August 9, 1920	mg. 0.83	mg. 0.83	mg. 0.82	mg. 0.61	mg. 0.66	mg. 0.80	mg. 0.86	mg. 0.27	mg. 0.41
	August 24, "	0.66	0.73	0.70	0.70	0.70	0.76	0.76	0.72	—
	September 7, "	0.70	0.63	0.15	0.66	0.93	0.74	0.68	0.88	0.56
	September 20, "	0.70	0.70	0.90	0.83	0.70	0.73	0.80	0.93	—
XANTHOPHYLL	August 9, 1920	1.60	2.00	1.60	2.20	2.00	2.00	1.80	1.90	1.00
	August 24, "	1.60	1.70	1.60	1.70	1.60	1.10	1.50	1.20	—
	September 7, "	2.10	2.70	3.50	1.40	1.90	1.80	2.10	2.40	1.30
	September 20, "	1.80	2.60	2.00	2.50	2.50	3.00	2.50	3.00	—
TOTAL CAROTINOIDS	August 9, 1920	2.43	2.83	2.42	2.81	2.66	2.80	2.66	2.17	1.41
	August 24, "	2.26	2.33	2.30	2.40	2.30	1.86	2.26	1.92	—
	September 7, "	2.80	3.33	3.65	2.06	2.83	2.54	2.78	3.28	1.86
	September 20, "	2.50	3.30	2.90	3.33	3.20	3.73	3.30	3.93	—
CHLOROPHYLL ($\alpha + \beta$)	August 9, 1920	13.8	12.8	11.8	12.8	12.6	14.8	12.6	11.6	7.9
	August 24, "	11.3	15.6	13.1	11.8	9.5	20.1	12.6	10.9	—
	September 7, "	14.4	14.8	12.7	11.0	15.2	12.7	14.5	16.2	13.6
	September 20, "	15.4	15.4	15.4	15.2	15.4	16.6	15.4	14.1	—

the chlorophyll was much less. This fact shows that the leaves should never be dried if accurate quantitative data are desired for the chloroplast pigments. This table shows also that certain fertilizers do not always produce certain effects upon the development of the chloroplast pigments. The effect then apparently is due to some factor which the fertilizer influences indirectly. Here a potash fertilizer produced plants high in chlorophyll while nitrogen produced plants relatively low in chlorophyll.

The experiments with potatoes, reported in this paper, show that high yields of tubers are correlated with heavy potash fertilization, which also is correlated with a low chlorophyll content of the leaves. Low yields of tubers are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves. High phosphate produces yields of potatoes which are less than those from the high potash plots and greater than those from the high nitrogen plots.

Conclusions

From the above experiments it is clear that only fresh leaves should be used in the determination of the four chloroplast pigments. A very large percentage of carotin and of xanthophyll is lost on drying. The process of drying the leaves is far more destructive to the carotinoids than it is to the chlorophylls.

Phosphorus, potash and nitrogen each may be correlated with an effect on the formation of the chloroplast pigments. Nitrogen was found to be correlated with an increase in the amount of chloroplast pigments which were present in each ten gram sample of fresh green leaves. The nitrogen was also correlated with an increase in the amount of the carotinoids. In potatoes, high potash evidently suppressed chloroplast pigment formation while this was also true in some of the plots of cotton. High phosphorus plots would then evidently produce more chloroplast pigments than potash and less than nitrogen.

Either cotton or potato plants fertilized with a mixture high in nitrogen always produces leaves with a high nitrogen content. Potato plants fertilized with a mixture high in potash produced leaves which contained the least potash, while cotton plants produced leaves which contained the most potash. Both cotton and potato plants which were fertilized with mixtures high in phosphorus also produced leaves which were high in phosphorus. In the case of potatoes the amount of sulphur in the leaves could not be correlated with the amount of nitrogen, potash or phosphorus while in the case of cotton the leaves high in nitrogen contained the least sulphur, and the leaves high in phosphorus contained the most sulphur.

Plots which were high in potash gave low yields of cotton while those high in nitrogen produced the most cotton per acre although this is not true

for all types of soil. High yields of potatoes are correlated with heavy potash fertilization which also is correlated with a low chlorophyll content of the leaves. Low yields are correlated with high nitrogen fertilization and a high chlorophyll content of the leaves.

When the results from a fertilizer experiment at Arlington Farm are correlated with results elsewhere it is seen that P, N, and K do not always have the same effect on the chloroplast pigments. At Arlington Farm the potash did not seem to suppress the chlorophyll formation nor did nitrogen increase chlorophyll formation, which is not in agreement with findings elsewhere.

The results given in this paper are in harmony with those of MAIWALD for potatoes. He found that potato leaves from plots fertilized by the addition of potash contained considerable less chlorophyll than leaves of potato plots to which no potash was added. The amount of the chlorophyll rapidly decreased in the potash plots from June to August. In the plots without potash the decrease in the chlorophyll was not so rapid. Leaves from plots fertilized with both potash and nitrogen showed a decided increase in their chlorophyll content. Plants in these plots did not show a decrease in chlorophyll from June to August as did the potash plots.

DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

LITERATURE CITED

1. CHIBNALL, ALBERT CHARLES. Diurnal variation in the total nitrogen content of foliage leaves. *Ann. Bot.* **37**: 511-518. 1923.
2. HENRICI, MARGUERITE. Chlorophyllgehalt und Kohlensäure-Assimilation bei Alpen- und Ebenen-pflanzen. *Verh. naturf. Ges. Basel* **30**: 43-136. 1919.
3. MAIWALD, K. Wirkung hoher Nährstoffgaben auf den Assimilationsapparat. *Angew. Bot.* **5**: 33-48, and 49-74. 1923.
4. PINCKNEY, R. M. Effect of nitrate applications upon the hydrocyanic acid content of sorghum. *Jour. Agr. Res.* **27**: 717-723. 1924.
5. REMY, TH., and LIESEGANG, H. Untersuchungen über die Rückwirkungen der Kaliversorgung auf Chlorophyllgehalt, Assimilationsleistung, Wachstum und Ertrag der Kartoffeln. *Landw. Jahrb.* **64**: 213-240. 1926.
6. RUSSELL, SIR JOHN. Soil improvement. Fertilizers and their uses. *Jour. Min. Agr.* **31**: 217-223. 1924.
7. SCHAEFER, ERNST. Studien über einige Staudeneigenschaften der Kartoffelpflanze. *Jour. Landw.* **72**: 163-195. 1923.

8. SCHERTZ, F. M. A chemical and physiological study of mottling of leaves. *Bot. Gaz.* **71**: 81-130. 1921.
9. ————. The quantitative determination of carotin by means of the spectrophotometer and the colorimeter. *Jour. Agr. Res.* **26**: 383-400. 1923.
10. ————. The quantitative determination of xanthophyll by means of the spectrophotometer and the colorimeter. *Jour. Agr. Res.* **30**: 253-261. 1925.
11. ————. The extraction and separation of chlorophyll ($\alpha + \beta$) carotin and xanthophyll in fresh leaves preliminary to their quantitative determination. *Plant Physiol.* **3**: 211-216. 1928.
12. ————. The quantitative determination of chlorophyll. *Plant Physiol.* **3**: 323-334. 1928.
13. URBAN, J. Über die Farbe des Rübenkrautes früh- und spätreifender Rüben. *Zeitschr. Zuckerind. Böhmen* **42**: 281-297. 1918.
14. VILLE, GEORGES. Recherches sur les relations qui existent entre la couleur des plantes et la richesse des terres en agents de fertilité. *Compt. Rend. Acad. Sci. Paris* **109**: 397-400, 628-631. 1889.
15. WEISSMANN, H. Über den Einfluss des Kaliums auf die Entwicklung der Pflanzen und ihren morphologischen und anatomischen Bau bei besonderer Berücksichtigung der landwirtschaftlichen Kulturpflanzen. *Zeitschr. Pflanzen-Düngung* **2**: 1-79. 1923.
16. WLODEK, JEAN. Recherches sur l'influence des engrais chimiques sur le coefficient chlorophyllien. *Bull. Acad. Polon. Sci. Let. Cl. (B) Sci. Nat.* **21**: 19-52. 1920.
17. ————. Recherches sur l'influence de la lumière et des engrais chimiques sur le coefficient chlorophyllien. *Bull. Acad. Polon. Sci. Let. Cl. (B) Sci. Nat.* **22**: 143-190. 1921.

THE EFFECT OF TEMPERATURE ON FLOWERS

F. M. ANDREWS

(WITH ONE FIGURE)

This paper is concerned with the opening and closing processes in the flowers of *Crocus* and *Tulipa*. As is well known from the work of PREFFER, the flowers of *Crocus* and *Tulipa* are only slightly photonastic. They are, however, extremely thermonastic. As regards this thermonastic response *Crocus* is much more sensitive than *Tulipa*. In the case of *Crocus*, the temperature may be so low that the flowers cannot open, yet the flowers may develop fully, nevertheless. Thus PREFFER found that in temperatures below 8° C. the flowers developed but did not open. He found that the flowers of some *Crocus* plants opened to an extent with fluctuations of only 0.5° C. He made this thermonastic movement more evident by means of a fine silver wire which projected beyond the flower division and which was fastened to the latter by means of lacquer. Others have found that a very fine glass capillary will serve this purpose equally well.

My experiments show that the flowers of one species of *Crocus* are capable of responding to a somewhat smaller temperature change than 0.5° C. This is the case with the flowers of *Crocus vernus* which has been found to be the most sensitive to temperature changes of any of the species of *Crocus* thus far investigated. To test this point a study of eight species of *Crocus* has been made. The "constant temperature room" has rendered excellent and trustworthy service, but it is not so easily nor so closely controlled as the device here described which has been constructed for this study. (See fig. 1.) This apparatus consisted of a bell-jar, A, of wide but low form, with an opening at the upper end for the insertion of a very sensitive thermometer, B, wires for the electric current, C, and a suitable stopper, D, for adjusting and holding these in place. A pair of bell-jars similar to the one just mentioned, the outer being somewhat larger than the other so as to cover the inner one, and held in place by a wire adjustment is even better under certain conditions. Or, an ordinary double-walled bell-jar serves the purpose very well where special precautions are necessary. In this case, however, provision must be made for the entrance of the electric wires under the base of the jar. In the low form of bell-jar shown in fig. 1, the electric wires encircle the interior at the bottom and are held in place by means of perforated perpendicular glass rods or posts, E. These glass supports are of such length as to allow several coils of number 20 nichrome heating wire, F, to be used. It was necessary to determine exactly before beginning the experiment what adjustments were required to raise and

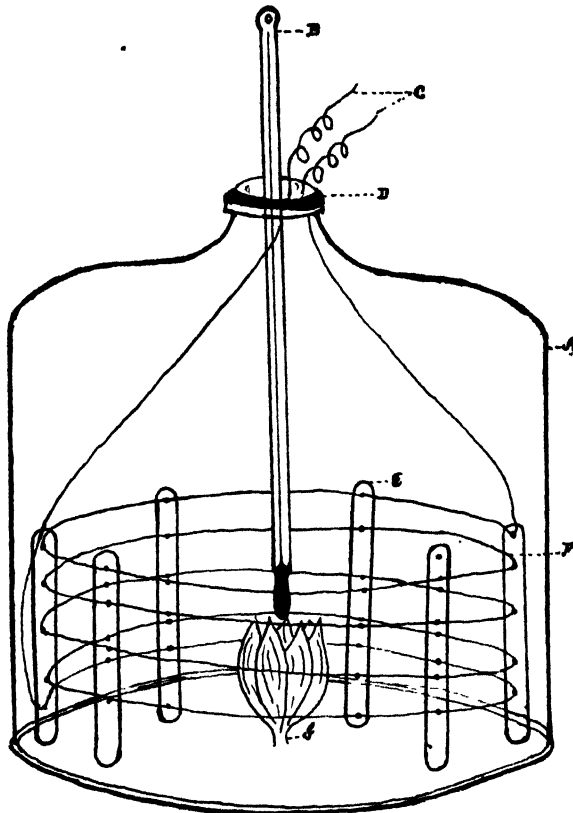


FIG. 1. Apparatus for demonstrating thermonastic responses of flowers.

maintain the temperature of the bell-jar to the desired point. With this apparatus the necessary heat for causing the opening movements of the flower segments of *Crocus* and *Tulipa* was readily produced by electricity. When the current was passed through the coils of nichrome wire, fig. 1, the necessary temperature was slowly or quickly produced according to the adjustment made. This was accomplished very exactly by using an electric current of 110 volts which was controlled by a lamp resistance in series with an adjustable wire rheostat. This arrangement also allowed the experiments to be performed either on potted plants, or better, on plants grown in the open and at any desired distance from the source of the current by increasing the length of the conducting wire. In this way the apparatus could be quickly placed over plants growing in flower beds. When it was observed that the flowers were in a probable condition for opening, the bell-jar, arranged as shown in figure 1, could often be placed over a considerable number of buds or flowers of *Crocus*. If the buds and flow-

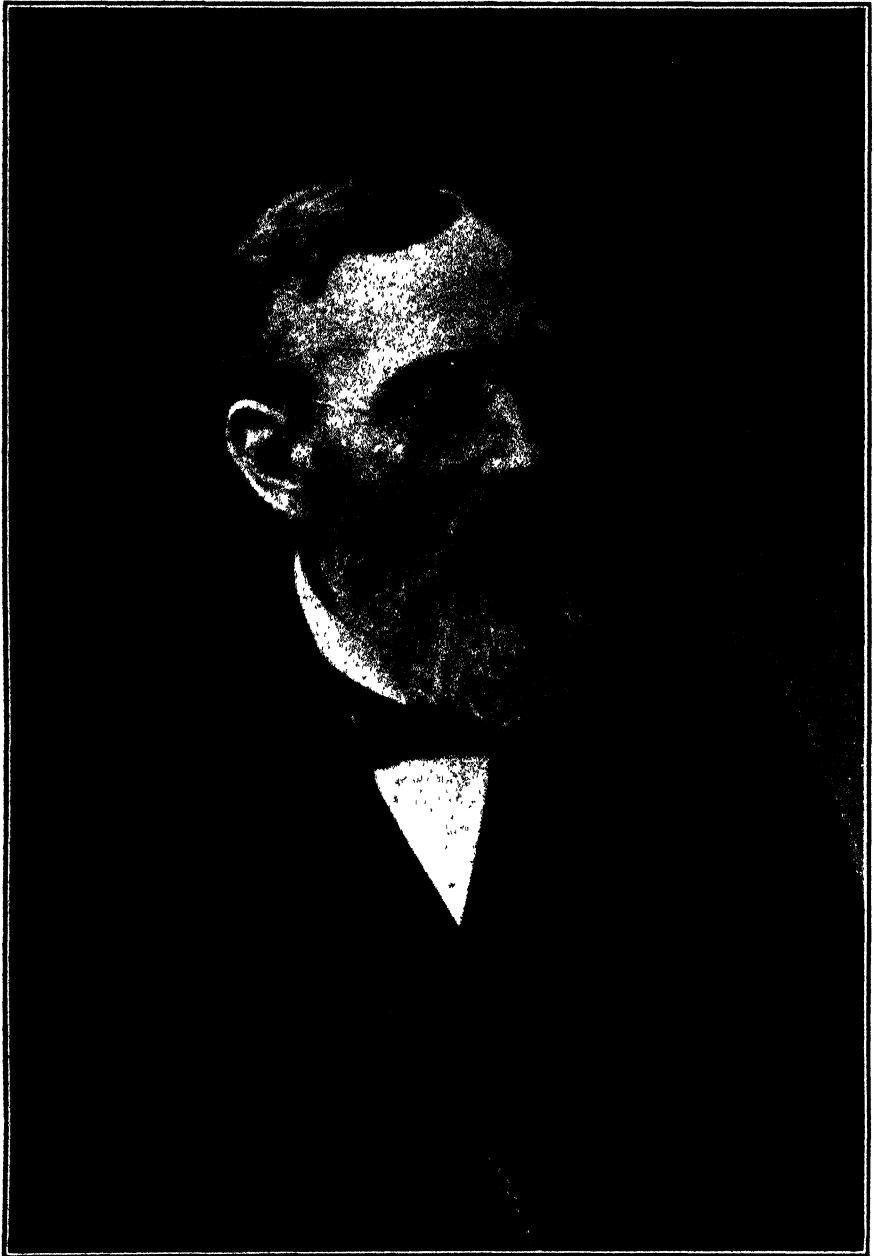
ers of *Crocus*, G, are in an active condition, they can be caused to open or close respectively 12 times in immediate succession by alternately warming, or cooling to an appropriate degree, suitable buds or flowers on cool days. In order to cover a considerable number of buds or flowers by the bell-jar, consideration should be given previously to the planting of *Crocus* for such experimentation. This plant will bear rather close setting of its corms without detriment.

For the study of the opening and closing processes in *Tulipa* much taller bell-jars than the one shown in fig. 1 must be used, and the wire arrangement for heating increased and placed on the interior of the bell-jar in accordance with the height of the flowers. Although, as stated, *Crocus* is only feebly photonastic, still some objection has been offered to certain experimentation carried out with potted plants of *Crocus* in a room. The apparatus here described, effectively removes this objection, since it brings to bear on the plants enclosed by the bell-jar only the temperature effect, and leaves the influence of light out of doors unaffected. The other *Crocus* plants in the experimental plot therefore, serve in this connection as controls. This apparatus when used on *Crocus* and *Tulipa* in the open has also the additional advantage that the plants are under somewhat more natural conditions in other respects than is the case with potted ones. By the use of this apparatus ROYER's idea that transpiration effects the flower movements is disproved, since this condition by my apparatus is controlled. These experiments are best carried out on cool days since then all the flowers out of doors are closed, while those that are in a responsive state under the electrically warmed bell-jar, can be caused to open. Caution should at all times be observed, to select flowers which have attained the proper stage of development. This is sometimes difficult to determine, or can be ascertained only by their failure to respond to the temperature changes. The flowers may be too immature, but more often they are too old and hence have passed, with respect to temperature, their perceptive condition. After a *Crocus* or *Tulipa* bud has been opened by warming under the bell-jar, then by removing the bell-jar or otherwise filling it with cool air, the open flowers will close. This occurs more quickly in *Crocus* than in *Tulipa*. The opening and closing of the flowers in both *Crocus* and *Tulipa* also take place rapidly or slowly according to the changes in temperature, within their responsive ranges. A slight fullness of the *Crocus* bud indicates a condition for opening, although this may be deceptive. Single flowers may be made to open very rapidly, if placed, as PFEFFER points out, in warm water after removal from a cool place. These flowers, however, rarely stand upright in the water, which makes some observations of their behavior less obvious. This difficulty may be overcome by enclosing the lower end of the perianth tube in a rather closely fitting glass tube of proper weight and

length, so that part of the glass tube's weight and lower end rests lightly on the bottom of a wide crystallizing dish in which the warm water is only a few cm. in depth. By this method a good many flowers may be observed simultaneously and the extreme opening process may be followed to good advantage.

By means of the electrically warmed bell-jar described here, flowers of *Crocus vernus* experimented upon out of doors were caused in many instances to open to an extent by increasing the temperature by only 0.2° C. The flowers of *Tulipa*, as is well known, are less responsive and no flowers of this plant were found that showed any tendency to open with a change of less than 1° C. In both of these plants the response that did occur in the flowers was much slower than when the temperature was higher or more favorable in the warmed air of the bell-jar or in the warm water. These experiments show that the flowers of both *Tulipa* and *Crocus* are much more sensitive to temperature changes than has been observed heretofore for these plants.

DEPARTMENT OF BOTANY,
INDIANA UNIVERSITY.



WILHELM PFEFFER
1845-1920

BRIEF PAPERS

WILHELM PFEFFER

(WITH ONE PLATE AND ONE FIGURE)

WILHELM PFEFFER was the son of an apothecary. He was born the 9th of March, 1845, in Grebenstein, near Cassel, Germany. He received his early education in Grebenstein and later attended the schools of Cassel.¹ He evinced in early life a wide and keen interest in various departments of science in which his information, even when yet a boy, was very unusual. At first, he was much interested in collections of various kinds. This work took him on many trips, especially in the Alps mountains. His investigations of the plants of this region led to the publication of his well known Bryographical Studies dating from 1867 to 1869.

As a university student PFEFFER's training was extensive. He studied at the university of Göttingen and received the degree of Doctor of Philosophy there in 1865. Afterward, he studied at the universities of Marburg, Würzburg, and Berlin. At Würzburg PFEFFER was a student of JÜLIUS VON SACHS. There he was associated with many other investigators who afterwards became distinguished workers in their fields: KLEBS, DE VRIES, GOEBEL, BREFELD, NOLL, STAILL, KRAUS, MÜLLER-THURGAU and many others. PFEFFER's preparation in physics and chemistry was very extensive, and the mastery of these sciences was of great service to him in his work in plant physiology.

As a teacher, PFEFFER's experience was also wide. He began his career formally as an academic teacher in the university of Marburg in 1871. He was professor in the university of Bonn in 1873; in the university of Basel in 1878; in the university of Tübingen from 1878 to 1887 and finally professor in the university of Leipzig from 1887 till his death, which occurred January 31, 1920.

His lectures were largely attended. On these he spent much time and frequently produced interesting and valuable demonstrations of plant activities by means of ingeniously contrived apparatus. Many of these experiments were extremely difficult to show to a large audience due to the special conditions which must not infrequently be provided.

In the laboratory, PFEFFER was careful and exacting. On the other hand his criticisms were always made in a most friendly and helpful manner. No work or trouble on his part was too great to help or encourage

¹ Some details in this paper have been taken or verified from Professor FITTING's account of PFEFFER. *Ber. deutsch. bot. Ges.* 38: 30-63. 1920.

any student who was industrious and determined to work. His great ability to understand and attack a problem in the best and most direct manner was astonishing, even to those of extensive training and experience. A very accurate memory nearly always enabled him to make an instant and exact reference to the needed literature. His keen insight and ability together with his long and successful experience gave him a grasp of the subject that was at once noted as unusual by all who came in contact with him.

PFEFFER's contributions to science number nearly one hundred. Among these are some papers which occupy comparatively few pages. However, what short papers he did write are very compact and to the point; for these, like all of his papers, teemed with new facts, to each of which he gave careful study. They frequently represented long periods of experimentation. In these papers, as elsewhere, he shows his ability to go directly to correct conclusions, and where necessary to summarize with great clearness and brevity. Other papers were much longer due to the nature of the topic under discussion. Many of his contributions were so extensive that they were published in book form. The second edition of his *Pflanzenphysiologie* is the largest contribution of this nature. PFEFFER's studies on osmosis (1877) are classical, and have had a wide bearing in many fields of science. The physico-chemical importance of PFEFFER's osmotic studies have been amply indicated by VAN'T HOFF in his well known studies of osmotic pressure, gas pressure and solutions. Mention should also be made of PFEFFER's *Physiologische Untersuchungen* (1873) which constitute one of his best known investigations. His studies of leaf movements are very extensive and cover a long period of investigation. The skillful methods used in these studies and the valuable results obtained constitute a very important advance in this field. His work on chemotaxis (1888) as well as his *Studien über Energetik* (1892) are of great interest and importance. These and many others of PFEFFER's valuable contributions to plant physiology could be mentioned if space allowed their enumeration. His ability to present and summarize the vital points of a subject is well shown in his *Pflanzenphysiologie* (1897-1904). In this work, a huge field of literature had to be carefully sifted and summarized and the results brought together in a connected account. The writing of this work was therefore an enormous task and it constitutes the greatest production of its kind in the field of plant physiology. It gives ample proof of PFEFFER's thorough grasp of the subject and shows his exceptional qualifications and his ability to successfully execute this difficult undertaking. Even if PFEFFER had written only his *Pflanzenphysiologie*, science would be deeply indebted to him, and PFEFFER, aside from any of his other distinguished work, would be entitled to a place of distinction.

In 1915 a "Festschrift" for PFEFFER was issued in honor of his seventieth birthday and the fiftieth year since he received his Ph.D. degree. This book also constitutes the 56th volume of the *Jahrbücher für wissenschaftlichen Botanik* of which PFEFFER was editor. After the death of his teacher, N. PRINGSHEIM, in 1895, PFEFFER, in conjunction with E. STRASBURGER, became editor of this well known journal. After the death of STRASBURGER in 1912 PFEFFER carried the burden of this task alone. This "Festschrift" is composed of papers written expressly for this volume by his former students in various countries. This volume also contains a list of PFEFFER's papers, beginning with his thesis which appeared in 1865, to and including the year 1915, although at that time his last book on sleep movements which appeared in 1915 is not listed. In this volume is also included a list of 260 of his former students. As a distinguished plant physiologist and as an organizer of the subject PFEFFER is unequalled. The nearest approach to him in these respects was JULIUS VON SACHS. This volume contains his photograph from which plate IV has been reproduced.

Aside from his regular work, he had some time to spend with his students. They were invited once each semester to his home. Moreover, he always presided at the botany club which held its meetings every two weeks, in the evening. In addition to the paper of the meeting the special privilege was granted to each person, before the meeting, to place in a box a botanical question for PFEFFER to answer. These questions he always answered, even without previous notice or preparation, without hesitation and very clearly and completely.

Fig. 1 is a photograph of the Botanical Institute where PFEFFER was director and professor. It was designed and build by A. SCHENK, the pre-

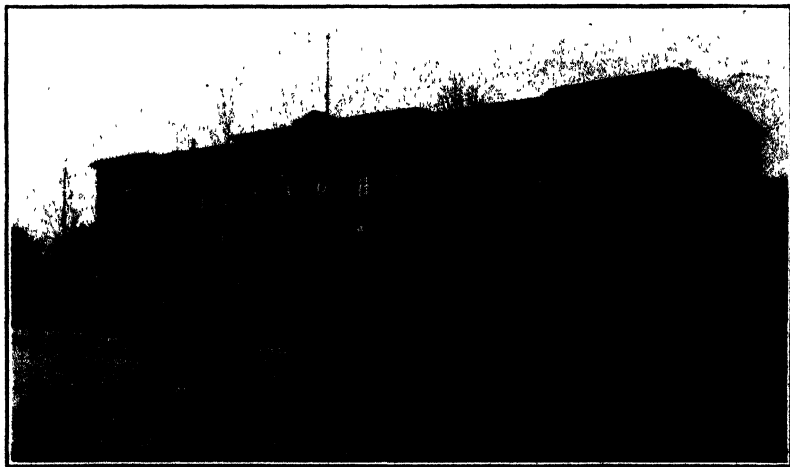


FIG. 1. Botanisches Institut, Leipzig.

decessor of PFEFFER as professor of botany at Leipzig. This photograph is taken from a paper written by Professor PFEFFER especially for the celebration of the 500th anniversary of the University of Leipzig.² It faces east which is the view shown here. It will be a familiar sight to those who have studied there. It will also give those who have not studied there an idea of the place from which so many of PFEFFER's important contributions to plant physiology have come. The building stands in the botanical garden. The basement of the building is chiefly used as special experimental rooms. The first floor is devoted exclusively to laboratories with the exception of the library and lecture room. The top floor serves as a residence for the Director. PFEFFER could take 20 research students at a time in this Institut. The fame of the Leipzig Institut was so unusual, with PFEFFER as its director, that it was necessary to make arrangements a long time in advance if one expected to secure a place. In addition there was generally a considerable waiting list. All of PFEFFER's many students, now scattered everywhere in so many different countries, count it a special privilege that they were fortunate enough to study under his direction. On account of PFEFFER's wide and successful career both as an investigator and a teacher he has done more than any one else to train and inspire the plant physiologist of the present day.—FRANK M. ANDREWS, *Indiana University*.

✓ A SIMPLE METHOD FOR NITRATE NITROGEN DETERMINATION IN WHEAT PLANTS¹

The greatest difficulty in getting satisfactory results in nitrate nitrogen determinations in plant material is due to the presence of the large amounts of soluble and colloidal organic substances, or to the difficulty in removing these substances before the determinations are made. When nitrates are determined by reduction and subsequent distillations, fairly large samples are necessary and the interference of the decomposition of the organic materials during the distillation makes the determination somewhat unsatisfactory. Likewise the colorimetric method is impossible unless these soluble organic substances are removed before evaporation. The removal of these substances would be equally desirable in the distillation method.

² This paper, entitled *Die botanischen Institute*, as well as the photograph used here, were presented to the writer by Professor PFEFFER.

¹ Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper no. 152, College of Agriculture and Experiment Station, State College of Washington, Pullman, Washington.

TABLE I
COMPARISON OF THE GILBERT AND SIMPLE METHOD OF DETERMINING NITRATE NITROGEN IN TWO VARIETIES OF WHEAT

VARIETY	GILBERT METHOD				SIMPLE METHOD		
	SAMPLE	NO ₃ N	AVERAGE	RECOVERED	NO ₃ N	AVERAGE	RECOVERED
Jenkins Club	gm. 0.2	mg. 0.3600 0.3600	mg. 0.3600	per cent.	mg. 0.3600 0.3600	mg. 0.3600	per cent.
Jenkins Club, plus 0.1 mg. NO ₃ N	0.2	0.4000 0.4000	0.4000	40	0.4600 0.4600	0.4600	100
Hybrid 128	0.2	0.0500 0.0550	0.0525		0.0500 0.0500	0.0500	
Hybrid 128, plus 0.1 mg. NO ₃ N	0.2	0.1000 0.0950	0.0975	45	0.1450 0.1350	0.1400	90

In an experiment where a large number of nitrate nitrogen determinations were to be made on eleven varieties of wheat in different stages of development, an attempt was made to select the simplest and most efficient method for removing the interfering organic substances preliminary to the phenol disulfonic acid treatment.

The method for clearing the solution, reported by BURRELL and PHILLIPS (1), in which lead acetate was used to precipitate the soluble matter, was tried but considered too long and somewhat difficult. The method used by GILBERT (2) was next tried in which carbon black is substituted for lead acetate, and was found much simpler for this purpose. The cleared solution in both methods contained a large amount of soluble salts which prolonged the evaporation of the solution. This was found to be caused by the addition of the magnesium carbonate. In order to eliminate the magnesium carbonate, the following method, which has proven to be very satisfactory, was evolved.

Two tenths gram of plant material which had been dried according to the method reported by THOMAS (3) was weighed out, transferred to a 250-cc. beaker and leached with 74 cc. of distilled water for an hour. After leaching, 5 cc. of saturated silver sulphate (AgSO_4) was added, followed by 1 cc. of normal copper sulphate (CuSO_4), 0.2 gm. of calcium hydroxide (Ca(OH)_2 powdery), and 2 to 3 gm. of carbon black. This extract containing the plant material was stirred after each addition of the above solutions and salts, and filtered. One half of the filtrate was evaporated to dryness, and nitrates determined by the phenol disulfonic acid method. The following table gives the nitrate nitrogen content of two varieties of wheat and the recovery of nitrates added to each variety as determined by the method used by GILBERT and the one here described.

Of the several varieties analyzed for comparison, only two are presented in the table. These were selected for their difference in nitrate nitrogen content and soluble organic matter content. The data show that both methods gave duplicate results on the plant material, but for some undetermined reason the recovery of added nitrates by the GILBERT method was incomplete, while in the modified method it was all recovered in Jenkins Club and nearly so in Hybrid 128.

The advantages of this simple method over that used by GILBERT are: (1) it reduces the filtrations to one; (2) it eliminates the washing of any residue; (3) it speeds up the evaporation of the filtrate; (4) it eliminates boiling; and (5) it gives good recovery to added nitrates. The above advantages make for speed and accuracy, both of which are important factors when a large number of determinations are to be made.—H. F. HOLTZ and CARL LARSON, *State College of Washington*.

LITERATURE CITED

1. BURRELL, R. C., and PHILLIPS, T. G. The determination of nitrate nitrogen in plants. Jour. Biol. Chem. **65**: 229-234. 1925.
2. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphorus and potassium in plant solutions. Plant Physiol. **1**: 191-199. 1926.
3. THOMAS, W. Nitrogenous metabolism of *Pyrus malus*. Plant Physiol. **2**: 55-70. 1927.

NOTES

Fifth International Botanical Congress.—At the time of the International Botanical Congress held at Ithaca in 1926, it was decided to hold the next congress in England. The announcement has been made that the fifth congress will be held at Cambridge, August 16–23, 1930. The meeting will be organized in seven sections, paleobotany, morphology and anatomy, taxonomy and nomenclature, plant geography and ecology, genetics and cytology, plant physiology, and mycology and plant pathology.

The chairman in charge of the program for plant physiology is Dr. F. F. BLACKMAN, of the Botany School at Cambridge. The papers to be presented will be arranged by invitation, and the discussion will also probably be arranged by the sectional subcommittee. Membership in the Congress is to cost one pound, which should be sent to the treasurer of the Congress, Dr. A. B. RENDLE, British Museum of Natural History, London, S. W. 7., not later than April 1, 1930. The treasurer desires early notification of the intention to attend the Congress, even if membership is not paid at once. The Congress will provide a rare opportunity to meet the plant physiologists of other lands.

Adoption of the Constitution.—The ballot on the adoption of the constitution resulted in a unanimous vote favorable to adoption, and the provisions become effective immediately. This instrument will enable the American Society of Plant Physiologists to handle its affairs advantageously, and lays a foundation for financial strength and stability. Plans are in preparation to place a printed copy of the constitution in the hands of all members of the society.

Representative on the A. A. A. S. Council.—Dr. P. D. STRAUSBAUGH, Professor of Botany at the West Virginia University, has been appointed by the President to represent the American Society of Plant Physiologists on the Council of the American Association for the Advancement of Science. He fills the vacancy in the representation caused by the death of Dr. HARRIS M. BENEDICT, of the University of Cincinnati, last October.

Increased Price of Volumes I and II of Plant Physiology.—The very large demand for the early volumes of PLANT PHYSIOLOGY has almost exhausted the editions printed during 1926 and 1927. The executive committee has considered the matter carefully, and has decided to increase the price for the remaining volumes for these two years to \$10 per volume. It is unfortunate that this step must be taken. The editorial board has tried to pursue a safe financial policy, printing editions no larger than could

be paid for rather promptly by the Society, but with what was considered an appropriate surplus of each issue. The unforeseen demand for the journal has depleted the surplus much more rapidly than was anticipated. While the few remaining volumes last, they can be had by addressing Dr. H. R. KRAYBILL, Department of Agricultural Chemistry, Purdue University, Lafayette, Indiana.

Portrait of Sachs.—In accordance with the announcement made in PLANT PHYSIOLOGY in October, 1928, a small number of portraits of JULIUS VON SACHS will be available for those who desire them for framing. There is something inspiring about the faces of great leaders, and laboratories decorated with the portraits of such men make a lasting impression upon students who are following in the footsteps of these forefathers of our science. The portraits of SACHS may be had in the same manner as the portraits of TIMIRIAZEFF, as announced on page 522 of Vol. 3 of PLANT PHYSIOLOGY.

Fourth Pacific Science Congress.—The fourth Pacific Science Congress, sponsored by the Pacific Science Association, is being held under the auspices of the Netherlands Indies Pacific Research Committee in Batavia and Bandoeng, Java, May 16–25, 1929. If it is possible to secure a report of this meeting, such matters as may interest plant physiologists will be presented to the readers of PLANT PHYSIOLOGY in some future number.

Seventh Colloid Symposium.—The seventh Colloid Symposium is to be held this year at Johns Hopkins University, Baltimore, Maryland, June 20–22, 1929. The guest of honor will be Dr. F. G. DONNAN, famous for his studies of colloidal equilibrium relations. Visitors expecting to attend this meeting should write to Dr. W. A. PATRICK, Department of Chemistry, Johns Hopkins University, for reservation of rooms in the University Dormitory. The number that can be accommodated in this way is limited to about 150, and cost of room and board for the meeting is but \$6.00. Visiting plant physiologists will find a cordial welcome at the Laboratory of Plant Physiology, also.

International Critical Tables.—The fifth volume of this important work has been published by the McGraw-Hill Co., N. Y. This volume contains data on viscosity of gases, metals and alloys, aqueous solutions of strong, weak, and non-electrolytes, and fluidity of non-aqueous solutions; kinetics of vaporization, solution, crystallization, diffusion and permeability; specific heat of gases and vapors, liquids, and solids; thermal effects, such as latent heat of fusion, vaporization, transformation, heat of adsorption

and wetting, heat of expansion, heat of mixing of liquids, heat of solution, precipitation, and dilution, heat of neutralization of acids and bases, heat of combustion of organic compounds, and heat of formation; thermal conductivity of gases, liquids, solids, and heat transfer by convection; radiometry (heat and light) including black body radiation, thermal radiation from selected sources, efficiency of selected sources of light, reflectivity of metallic and non-metallic substances, transmissivity, spectral absorption of light and heat by inorganic and non-metallic materials, and spectral filters; spectroscopy, including emission spectra, persistent lines, absorption spectra (visible, ultraviolet and infra-red spectra), solar spectrum, celestial spectra, low temperature luminescence, fluorescence of gases, spectral series of elements, band spectra, etc.; photometry; mechanical equivalent of light; properties of photographic materials; and properties of soaps and their aqueous solutions.

This volume is without question one of the most valuable for physiologists in the entire set. It forms an invaluable source of information for biophysical research.

The National Research Council has announced that it is impossible to present the entire work in the five volumes originally planned. This is due to the extraordinary expansion of scientific investigations during and since the late world war. Faced with the alternatives of eliminating data from unpublished chapters, or eliminating whole chapters, or expanding the work, the decision was reached to expand the work to seven volumes. Through the generosity of the Research Corporation, the Chemical Foundation, and the United States Steel Corporation, enough of the cost of the additional volumes was met to permit offering them to all subscribers at the same price per volume as the original pre-publication subscription price, \$7.00 per volume. This makes the whole work to subscribers \$49.00 instead of \$35.00 as announced previous to the publication of the first volume. While it is unfortunate to have to change the price of the complete set to those who subscribed for it at the lesser price, it is surely wiser to increase the size and cost of the work than to eliminate any of the assembled data.

Volume six will contain the data for X-rays, electronics, electricity, electrical conductivity, electrolytic E.M.F., and magnetism. The final volume will present data on sound, refractivity, optical activity, chemical kinetics, thermodynamics, explosives, and will contain a complete index.

Physiology and Biochemistry of Bacteria.—This work by Prof. R. E. BUCHANAN and Dr. ELLIS I. FULMER of the Iowa State College is being published by the Williams and Wilkins Co., Baltimore, Md. Vol. I contains five chapters as follows: Introduction and scope of physiological bacteriol-

ogy; growth phases and growth rates in cultures of microorganisms; chemical composition of the cells of microorganisms; physico-chemical and physical characteristics of microorganisms and their environment; and energy relationships, growth and movement of microorganisms. Energetics.

The chapter on growth rates presents the facts and theories, with attention to the autocatalyst theory of ROBERTSON, but takes the sensible view that plotted curves and equations describing them are not to be used without caution in attempts to interpret the causal relationships of growth. In the higher plants we certainly have better theories than the autocatalyst theory to explain sigmoid growth curves.

The chemical composition chapter gives data on water content, elementary constitution, organic constituents, carbohydrates, fats and other lipoids, pigments, and inorganic globules, crystals and inclusions.

The final chapter considers types of energy, sources of energy for microorganisms, utilization of energy in synthesis, heat production, light production, and movement.

The work fills a real need, bringing together much valuable and scattered data. It calls attention to the gaps in our knowledge, and should stimulate investigators to more vigorous attack upon the unsolved problems of bacterial life. It repays careful reading. The price of the volume is \$7.50.

Enzyme Actions and Properties.—The translation by R. P. WALTON of the treatise on Enzyme Actions and Properties by ERNST WALDSCHMIDT-LEITZ makes available in English the first comprehensive and authoritative account of the studies of the WILLSTÄTTER school on the purification and measurement of enzymes, studies in which the author played a prominent rôle. The book is divided into two parts. The first part deals with the general mathematical and physico-chemical factors,—the colloidal and electrolyte nature, specificity, reaction kinetics, activation, and methods of preparation, purification and determination of enzymes. Here WILLSTÄTTER's adsorption methods are fully discussed.

In the second part a few typical and much-studied enzymes are taken as types of the various groups, and are considered in great detail. Castor bean lipase, malt amylase, and yeast sucrase are the plant enzymes which are treated comprehensively. In general, animal enzymes get much more attention, which only emphasizes how much more is known about them than about plant enzymes. However, quite a little English and American work seems to have been overlooked, although these omissions affect only the specific and not the general value of the work. The translator has done a good job, and usually has made smooth English. However, there is no

justification for the use of such spellings as "peroxydase" and "oxydase," or the consistent failure to capitalize generic plant names. The format is good, and the author and subject indices comprehensive. The book is 255 pages, is published by John Wiley and Sons, and retails for \$4.00.

Hydrostatic-Pneumatic System of Trees.—An excellent monograph on sap ascent has been published by the Carnegie Institution as Publication no. 397, under the title, "The hydrostatic-pneumatic system of certain trees: Movements of liquids and gases." The authors are Dr. D. T. MACDOUGAL, J. B. OVERTON, and GILBERT M. SMITH. They discuss the liquid and gas system of species of *Salix*, *Quercus*, *Alnus*, *Juglans*, *Pinus*, and others, and give a fine presentation of the organization of woody stems. This section on organization should be the possession of every student of plant physiology. Then follow sections on the path of the transpiration stream, factors affecting localization of the stream, variations in the path, and lateral movement of water in the stems.

The later sections deal with the gas system of the woody stem, suction and pressures and the factors affecting them, and the rôle of living cells in sap flow. The conclusions are in agreement with DIXON's cohesion theory of sap rise, and give no comfort to those who are following BOSE's interpretation of sap rise. The price of this publication is \$1.25 (cloth \$2.25), and orders should be sent to the Carnegie Institution, Washington, D. C.

Colloid Chemistry.—A second edition of THE SVEDBERG's Colloid Chemistry has been published by the Chemical Catalog Co., New York. This edition gives more space to the X-ray method of analysis of colloidal sols and gels than the first edition. Improvements are recorded in the technique of ultramicroscopy, and measurements of cataphoresis and diffusion. Also, the chapter dealing with the DONNAN potentials (Part III) has been revised in accordance with the recognized importance of the DONNAN phenomena. The book is quoted at \$5.50. Orders should be sent to the publishers:

Growth.—A little book from the Yale University Press by Dr. WILLIAM J. ROBBINS, SAMUEL BRODY, ALBERT GARLAND HOGAN, CLARENCE MARTIN JACKSON, and CHARLES WILSON GREENE considers the nature of growth, its statistical, nutritional and morphological aspects, and physiological regulation of growth. The book is a series of popular lectures, mainly on animal growth, presented before the Missouri chapter of the Sigma Xi. The price is \$3.00.

PLANT PHYSIOLOGY

JULY, 1929

ON SOME LIMITING FACTORS IN THE USE OF SATURATED PETROLEUM OILS AS INSECTICIDES¹

HUGH KNIGHT,² JOSEPH C. CHAMBERLIN,²
AND CHAS. D. SAMUELS

(WITH TWO FIGURES)

Introduction

Owing to the increased use of saturated petroleum oils (*i.e.*, petroleum oils from which all, or nearly all *unsaturated hydrocarbons* have been removed, usually 98 per cent. or more) as insecticides when applied to foliage-bearing fruit-trees, it has become important to study the effects thus produced on the host plant.

The insecticidal efficacy has already been established by DE ONG and the two senior authors of this paper (2). It was noted that these oils apparently produce some adverse physiological effects on citrus trees, but this phase of the problem was not especially investigated.

The general nature of the deleterious effects accompanying the use of *heavy*, white-oil sprays has recently been described by WOGLUM, LA FOLLETTE and LANDON (6) as follows:

“The bad effects which have been noted on oranges in some degree since highly refined heavy oil sprays have been used are numerous. They include retarding of blossoming; reduction of blossoming; reduction of crop; retarding fruit coloration; interfering with normal sweat room coloring; drop of immature fruit; drop of mature fruit; roughened texture; mummifying of fruit; increasing crystallization; producing insipid flavor;

¹ The investigation reported as Part I of this paper was conducted by KNIGHT and SAMUELS, Part II by KNIGHT, and Part III by CHAMBERLIN. The work was done while the authors were in the employ of the California Spray-Chemical Company, of Watsonville, California. The studies reported on in Part III were made independently.

² Formerly Assistants in Entomology, Citrus Experiment Station, University of California, Riverside, California.

decrease in acidity, soluble solids and sugars; increase of dead wood; occurrence of fruit burn and leaf drop; difficulty in cleaning fruit, and "gumming-up" packing house conveyors. . . . Some of these troubles were costly to the grower. For instance extreme application of the heaviest oil sprays on Valencias during October and November, in some cases reduced the succeeding crop as much as 50 per cent., and affected the quality of the fruit then on the trees."

Not all of these effects have been fully verified. Some of them are established in the discussion which follows. It should be emphasized that these effects result primarily from the application of *heavy oils* (i.e., saturated lubricating oils of high viscosity).

The quick-breaking white oil sprays were developed particularly with reference to California citricultural conditions and it is there that they have been most extensively used. As insecticides they are markedly successful and because of the decreasing efficacy of HCN fumigation in California due to the development over wide and increasing areas of HCN resistant scale (5), heavy oil sprays have rapidly come into use. The practical need of a substitute for present methods of HCN fumigation is not likely to lessen, but the results herein presented indicate that this substitute should be something other than a *heavy* petroleum oil spray. Our data emphasize the extreme importance of a knowledge of the metabolic and pathological results following the use of oil sprays.

Part I

SOME NOTES ON THE PHYSIOLOGICAL EFFECTS OF SATURATED, WHITE PETROLEUM OILS ON CITRUS

In order to ascertain something of the metabolic changes induced in the plant by the application of petroleum oil as insecticides, the following experiments were made. The problem was attacked from three angles, namely transpiration, photosynthesis and respiration. The plants used were "seed-bed stock" of sour orange (*Citrus aurantium* Linn.).

TRANSPIRATION.—The plants for the transpiration tests were taken from two nurseries, one in Riverside, California, and one in Glendora, California, and were about 25 centimeters in height. Preliminary tests showed that the source of the plants is important. Those from the Riverside nursery taken in August had an average weekly transpiration rate of 40 grams of water per square decimeter of leaf surface, while plants, apparently just as thrifty, taken from Glendora, where cultural conditions were not the same, gave a weekly average of 21 grams per same unit area. Plants from the Riverside nursery taken in February showed a transpiration rate of 12.5 gm. per week. This indicates that the transpiration rate varies greatly with differences of environment—cultural, climatic and seasonal.

Two sets of experiments were run, the first between August and October, 1926; the second between February and May, 1927. The data are presented in tables I and II. The experimental conditions, aside from season, were identical in the two cases. The plants were grown in sealed jars containing HOAGLAND's nutrient solution. The weekly transpiration losses were replaced with distilled water. The leaf surface of each plant was measured and the loss of solution determined by daily weighings. The average transpiration loss for the two weeks prior to the application of the oil was taken as a norm for purposes of comparison. All results are expressed in two forms: first, in grams of water transpired per week per square decimeter of leaf surface; and secondly, as percentage of the "normal" rate as determined prior to treatment. Individual variations between plant and plant are considerable and differences of less than fifteen to twenty per cent. can not be considered significant. The data presented in any one vertical column are comparable, but those in different vertical columns are not, owing primarily to fluctuations in humidity.

Both humidity and temperature records were kept for the first test (table I). They are not included for the reason that no significant correlation could be found. Within the normal range of variation temperature alone is not significant, for, if the ratio of relative humidity to temperature remains constant, there is no significant change in transpiration. When the relative humidity drops, however, there is an immediate increase in transpiration. But since the change in transpiration caused by weather conditions was constant for all lots included in the tests, it may be disregarded in the present connection.

The application of oil causes a sharp and abnormal drop in the transpiration rate, the extent of the disturbance varying directly with the viscosity of the oil. In the case of kerosenes and light lubricating oils, recovery is effected in from one to three weeks. With saturated oils of high viscosity (100–110 seconds SAYBOLDT) recovery does not begin under six or seven weeks and may be delayed much longer than that. The length of time required for recovery is also correlated with the amount of oil applied. It is more rapid where the oil has been applied in small amounts (*i.e.* when applied as an emulsion), than where it is applied in large amounts (*i.e.* painted on in pure form). As indicated by experiment no. 12, table I, the presence of even a small percentage of unsaturates in an oil is sufficient to cause a sharp drop in the transpiration rate and to postpone recovery materially.

PHOTOSYNTHESIS AND RESPIRATION.—To determine the extent to which photosynthesis was affected by an application of oil, a thrifty potted sour orange plant somewhat more than 60 centimeters in height was kept in a dark chamber for fifteen days, when the leaves gave no further starch

THE EFFECTS OF PETROLEUM OIL ON THE TRANSPIRATION OF SOUR ORANGE NURSERY STOCK (AUGUST TO OCTOBER, 1926)

No. of EXPERIMENT	GENERAL SPECIFICATIONS OF OIL ¹	MODE OF APPLICATION	TRANSPIRATION RATE AT START OF TEST ²	TRANSPIRATION RATE PER WEEK AFTER APPLICATION OF OIL WEEKS NUMBERED 1-8 ³							
				1	2	3	4	5	6	7	8
1	Saturated; white petroleum; viscosity 106	Both sides of leaf painted with pure oil	38.9 gm. per week 100 per cent.	15.3 gm. 39 per cent.	15.7 gm. 40 per cent.	18.4 gm. 47 per cent.	14.3 gm. 37 per cent.	12.3 gm. 32 per cent.	12.6 gm. 32 per cent.	18.1 gm. 47 per cent.	22.3 gm. 57 per cent.
13	Same as above	Same as above	46.7 gm. per week 100 per cent.	12.2 gm. 26 per cent.	28.0 gm. 60 per cent.	25.7 gm. 55 per cent.	30.8 gm. 66 per cent.	31.3 gm. 69 per cent.	38.0 gm. 81 per cent.	45.3 gm. 97 per cent.	—
11	Saturated; white petroleum; viscosity 50	Same as above	34.4 gm. per week 100 per cent.	27.7 gm. 81 per cent.	27.2 gm. 79 per cent.	31.2 gm. 91 per cent.	27.7 gm. 81 per cent.	—	—	—	—
6	Same as above	2 per cent. quick-breaking emulsion	30.1 gm. per week 100 per cent.	16.1 gm. 54 per cent.	35.7 gm. 119 per cent.	—	—	—	—	—	—
9	Highly refined nearly saturated kerosene	Both sides of leaf painted with pure oil	43.5 gm. per week 100 per cent.	29.8 gm. 68 per cent.	51.3 gm. 118 per cent.	44.0 gm. 101 per cent.	—	—	—	—	—
12	80 per cent. saturated; lubricating petroleum; viscosity 45	Same as above	31.5 gm. per week 100 per cent.	12.3 gm. 39 per cent.	18.1 gm. 57 per cent.	18.8 gm. 60 per cent.	11.0 gm. 35 per cent.	15.6 gm. 50 per cent.	11.4 gm. 36 per cent.	9.8 gm. 31 per cent.	8.1 gm. 25 per cent.
10 ⁴	None	Check; no treatment	34.2 gm. per week 100 per cent.	—	—	39.2 gm. 114 per cent.	49.0 gm. 143 per cent.	40.2 gm. 117 per cent.	29.4 gm. 86 per cent.	37.6 gm. 109 per cent.	21.7 gm. 63 per cent.

¹ Degrees of saturation and viscosities are fairly close approximations. Viscosities are given in seconds SAYBOLT at 100° F.² Average rate as determined by two weeks' observation, in grams of water per square decimeter of leaf surface per week.³ Percentages to nearest whole per cent. only.⁴ Owing to an accident in setting up, there are no readings for the check for the first two weeks following the oil treatments.

TABLE II
THE EFFECTS OF PETROLEUM OIL ON THE TRANSPORTATION RATE OF CITRUS (SOUR ORANGE) NURSERY STOCK (FEBRUARY TO MAY, 1927)¹

NO. OF EXPERIMENT	GENERAL SPECIFICATIONS OF OIL	MODE OF APPLICATION	TRANSPIRATION RATE AT START OF TEST	TRANSPARATION RATE PER WEEK AFTER APPLICATION OF OIL. WEEKS NUMBERED 1-8							
				1 ²	2	3	4	5	6	7	8
21	Saturated; white petroleum; viscosity 106	2 per cent. quick-breaking emulsion	10.4 gm. 100 per cent.	2.0 gm. 19 per cent.	2.4 gm. 23 per cent.	4.5 gm. 42 per cent.	9.1 gm. 87 per cent.	6.1 gm. 59 per cent.	7.0 gm. 62 per cent.	15.8 gm. 152 per cent.	----- -----
26	Same as above	Same as above	16.7 gm. 100 per cent.	5.9 gm. 35 per cent.	4.2 gm. 25 per cent.	5.6 gm. 33 per cent.	9.8 gm. 59 per cent.	7.0 gm. 42 per cent.	8.4 gm. 50 per cent.	21.0 gm. 125 per cent.	----- -----
24	None	Check; no treatment	11.8 gm. 100 per cent.	7.3 gm. 62 per cent.	14.0 gm. 118 per cent.	13.3 gm. 112 per cent.	11.7 gm. 99 per cent.	10.5 gm. 89 per cent.	12.6 gm. 107 per cent.	9.8 gm. 83 per cent.	13.6 gm. 115 per cent.
37	None	Check; no treatment	15.7 gm. 100 per cent.	8.1 gm. 52 per cent.	16.3 gm. 104 per cent.	16.6 gm. 106 per cent.	14.6 gm. 93 per cent.	11.7 gm. 74 per cent.	15.9 gm. 101 per cent.	11.1 gm. 71 per cent.	15.3 gm. 97 per cent.

¹ See footnotes 1, 2, 3, table I, which likewise apply to the data in this table.

² Initial low transpiration during the first week following treatment due to humid weather. Note figures for checks.

reaction to the iodine test. In the evening of the fifteenth day the plant was placed in very subdued light and was treated as follows:

Several leaves were painted on both surfaces with (1), a saturated white oil of 106 seconds viscosity; (2), with a saturated white oil of 50 seconds viscosity; and, (3) with a nearly saturated, highly refined kerosene oil. The treated leaves were then tagged and the plant returned to the dark chamber till the following evening when it was put outside, hence 36 hours lapsed before exposure to sunlight.

Sections were cut from the treated leaves daily and tested for starch. It was not until the fourteenth day that those of lot 1 gave a faint reaction to starch, and they *had not returned to normal on the fortieth day when the test was terminated*. Lot 2 showed a faint starch reaction on the first day and had returned to normal by the fourth. Lot 3 gave starch reaction the first day. Checks gave a normal starch reaction the first day.

In order to determine the extent to which photosynthesis was interfered with under orchard conditions, analyses of the starch content of leaves taken from a lemon grove which had been sprayed with a white, saturated petroleum oil emulsion (viscosity 106 seconds SAYBOLDT) at 2 per cent. concentration, were made at approximately regular intervals. The results are set forth in table III. These lemon trees were sprayed on December 15, 1926. Surrounding lemon groves were fumigated. Leaf samples were taken at random from both the sprayed and fumigated trees, those from the fumigated trees being taken at the beginning and at the end of the experimental period. These latter may be considered as a check. The

TABLE III

STARCH ACCUMULATION IN LEMON LEAVES SPRAYED WITH A SATURATED WHITE
PETROLEUM OIL SPRAY
(VISCOSITY 106)
(FIELD CONDITIONS)

NATURE AND DATE OF TREATMENT	GRAMS OF STARCH IN 5 GRAMS OF LEAVES	PER CENT. OF STARCH TO DRY WEIGHT OF TREATED LEAVES	DATE SAMPLED
	<i>gm.</i>	<i>per cent.</i>	
Fumigated with hydrocyanic acid gas, December 15, 1926	0.0986	1.97	Dec. 29, 1926
	0.0960	1.92	Feb. 25, 1927
Sprayed with white oil emulsion at 2 per cent. concentration, December 15, 1926	0.0910	1.92	Dec. 29, 1926
	0.0744	1.49	Jan. 12, 1927
	0.0566	1.13	Jan. 21, 1927
	0.0604	1.21	Feb. 8, 1927
	0.1160	2.32	Feb. 25, 1927

results show that the starch content of the unsprayed trees remained constant within the limits of normal variability and experimental error. In the sprayed block the starch content dropped off markedly the first five weeks, and then rose rapidly until at the termination of the test it was far in excess of that of the check trees. Results similar to the above, showing increased starch content in the leaves two or more months after spraying have been noted in several other groves.

An attempt was also made to determine the effect on respiration, by the application of a heavy white oil as follows:

The above-ground portion of a potted Citrus plant was sealed under a large bell-jar. A small opening was provided at the bottom for the inflow of air, and a second small opening at the top to permit samples to be drawn off. The air taken out at the top was run through a gas train which removed the moisture and then the carbon dioxide was trapped in soda-lime and weighed. The amount of air passing through the system was controlled by means of an aspirator connected to the train. In this way the volume of water flowing from the aspirator was equal to the volume of air entering the system. Thirty-six liters of air were drawn through the apparatus for each determination. Several determinations were made with the empty apparatus to determine the amount of CO_2 normally present in the air. This was found to be 0.0166 grams per 36 liters. It is evident that when the same amount of air is drawn through the apparatus with the plant enclosed and more than this amount of CO_2 is found, that the plant is giving off CO_2 , or, in other words is respiring in excess of photosynthesis. The point at which these two quantities are equivalent is indicated by line "A" in fig. 1, where the results of this test are shown in graphic form. The distance of the plotted curve above or below this line therefore shows the extent to which the one or the other of these processes predominates.

Two thrifty untreated Citrus trees about six decimeters in height were placed under bell jars as before indicated and the CO_2 value was determined for each on four consecutive days. The curve (fig. 1) is well below line "A" and shows that photosynthesis was therefore in excess of respiration, the plants fixing an average of ten milligrams of CO_2 each day during the period covered by the test. Each run lasted approximately five and one-half hours. The four-day average is indicated in fig. 1 by the broken line designated "Norm." This is to be considered an average of photosynthetic activity for the plants in question, only for this season of the year; it would probably be greatly exceeded during the growing season.

The plants were then treated as follows: The entire leaf surface of one was painted with saturated oil of 106 seconds viscosity. The other was sprayed with a 2 per cent. emulsion of the same oil.

The graph, fig. 1, is self-explanatory. Respiration was enormously increased, the plants evidently oxidizing great quantities of reserve food.

Neither plant had returned to normal at the expiration of a month after the application of the oil. The reaction of the two plants differed only in degree, as was to be expected.

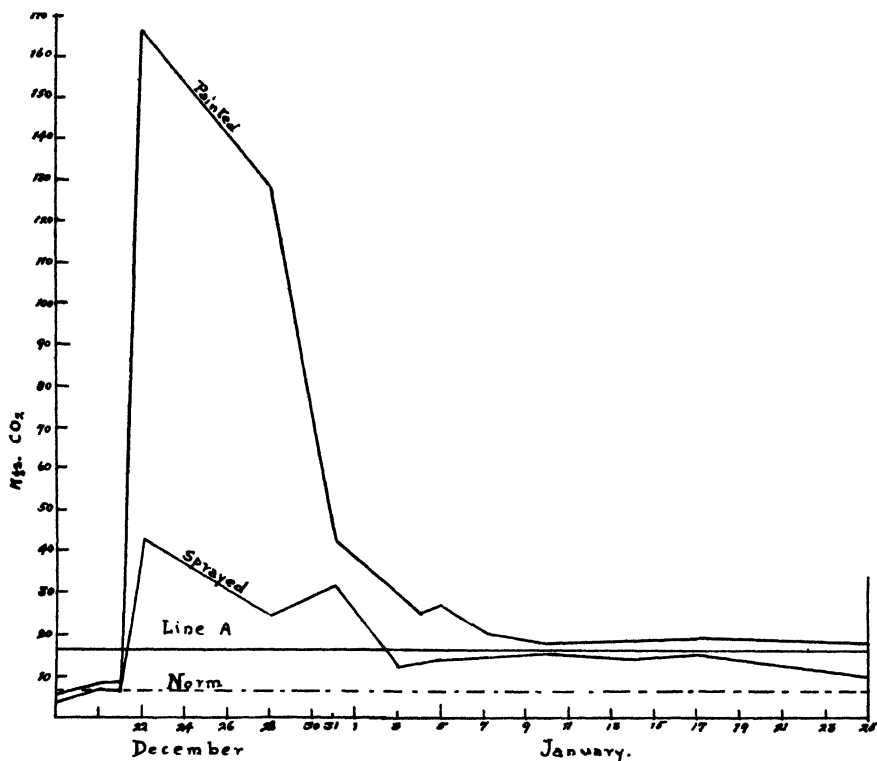


FIG. 1. Effect of oil sprays on respiration of Citrus trees.

It is recognized that the data presented are not sufficient to furnish a basis from which to draw definite conclusions. They indicate, however, that the application of a viscous, saturated petroleum oil induces profound metabolic changes in Citrus trees which persist for an extraordinary length of time.

It seems desirable to emphasize the fact that mere lack of "corrosive" qualities or the possession of "neutrality" or "chemical inertness" on the part of an oil does not imply that it is without deleterious effects when applied to living plants.

Part II

THE TRANSLOCATION OF PETROLEUM OIL IN THE LIVING PLANT (CITRUS)

Part I of this paper presented in a general way some of the physiological effects produced on the metabolism of *Citrus* by an application of a highly

refined, saturated petroleum oil, and indicated that the inhibitory effects are of surprisingly long duration.

Field observations also indicate that the heavier oils are absorbed by the leaves and remain therein for a very long period of time. The experiments already presented show that the functional activity in the leaf is partially resumed within about sixty days. In order to ascertain, if possible, how the oil was finally disposed of by the plant a histological study of oil penetration into the leaf was undertaken. To accomplish this successfully it was necessary to devise a special technique for staining the oil within the leaf tissue. It was recognized at the outset that none of the solvents in general use could be employed, as they are all oil solvents; and the problem was to fix or clear the leaf without dissolving the oil. Consequently the use of alcohol, xylol, or any of the essential oils as well as canada balsam was precluded, as was also the paraffin method of preparing sections.

Two kinds of preparations were desirable, (1) a flat gross preparation in which the distribution of oil in the leaf could be observed over a relatively large area, and (2), cross-sections in which the individual cells could be observed in their relation to the absorbed oil thus securing some insight into the penetration of oil into the cells themselves.

For the gross preparations it was necessary to "clear" the leaf, that is, to dissolve the chlorophyll and render the leaf transparent without dissolving or disturbing the oil; and for cross-sections, to fix the tissues for staining and sectioning also without disturbing the oil.

A short note in "Stain Technology" (4) gave a suggestion which materially helped to solve both phases of the problem. This note called attention to the use of pyridine in aqueous solution as a carrier for a fat stain. Pyridine is soluble in water as well as oil. Both Sudan III and IV, and Oil Soluble Red O are soluble in pyridine. When an oil-treated leaf is immersed in a saturated solution of Oil Soluble Red O dissolved in an aqueous pyridine solution, the stain, having a greater affinity for the oil than for the aqueous pyridine solution, is taken up by the oil. The solution of pyridine can then be removed by washing. In addition it was found that an aqueous solution of pyridine is an excellent solvent for chlorophyll, thus permitting "the killing of two birds with one stone" for it thereby became possible to clear the leaf and stain it at the same time without disturbing the oil.

The technique developed is substantially as follows:

For flat gross preparations.—Immerse in sixty per cent. aqueous solution of pyridine. Heat over water-bath. When discolored pour off and refill with fresh solution. Repeat (usually twice is sufficient) till solution remains clear and specimen becomes transparent.

Immerse for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. pyridine.

Wash in running water.

Pass through (1) glycerine-water (equal parts), followed by (2) pure glycerine.

Clear in carbol-glycerine (1 part carboic acid to 2 parts glycerine). Heat gently and watch carefully under dissecting microscope till clear. Specimen should be turned under-side up, when oil droplets can be seen distinctly.

Pass through glycerine again and mount in glycerine-jelly.

Allow to harden and seal with clear "Duco."

For cross-sections.—Fix in chrome-acetic acid for 48 hours.

Wash in running water.

Immerse in 5 per cent. formalin for 30 minutes.

Wash again in running water.

Immerse in 50 per cent. pyridine (aqueous solution) for 10 minutes.

Stain for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. aqueous solution of pyridine until color ceases to stream (watch carefully).

Wash in running water.

Section. See below.

Pass through (1) glycerine-water, followed by (2) glycerine.

Mount in glycerine-jelly and seal with clear "Duco."

Inasmuch as imbedding in paraffin is precluded, cross-sections were made by means of pith or cork. The freezing method could no doubt be used, but the writer has not had the opportunity of trying it. The operations of staining, washing, clearing and so on, are carried on with the aid of watch-glasses or small shell vials. If watch-glasses are used for staining they should be placed in closed petri dishes to prevent excessive evaporation. Watch-glasses are preferable for use with cross-sections. Staining may be done either before or after sectioning. If done before, shell vials will be found preferable.

For purposes of comparison the following oils were used:

(1) Heavy, saturated, white petroleum oil of 100–110 seconds viscosity SAYBOLDT at 100° F.

(2) A medium lubricating oil consisting of an equal mixture of 1 and 3. Saturation about 97 per cent.; viscosity about 67.

(3) Light, lubricating petroleum oil of about 50 seconds viscosity and 96 per cent. saturation.

(4) Light, lubricating oil of 44 seconds viscosity and 67 per cent. saturation; an unsaturated oil probably blended with a distillate.

It is regrettable that accurate data regarding other physical constants of these different oils are not available, such as vapor pressure at ordinary temperature, acidity, rate of oxidation, etc. Until such information is available much of the value of this type of study is lost.

EXPERIMENTAL DATA

Four potted orange plants were selected, of uniform size (about 6 decimeters in height) and having one or two lateral branches. Several of the leaves, from the tip to center of one limb, were carefully treated with oil applied by means of a small artist's brush, the oil being applied to both surfaces of the blade as far back as the petiole. The limb was tagged at the base of the lowest treated leaf. After treatment the plants were set away in the lath house.

Oil number 1 was taken as an index for the initial penetration tests, samples being cut from the leaves at intervals of 1, 2, 4, 8 and 24 hours. Owing to the great amount of labor involved, it was not possible to take samples from all four treated plants for this initial test. This is unimportant, the only possible difference being one of rapidity of penetration, the lighter oils penetrating more rapidly. Oil number 3, for example, disappeared from the surface in approximately three days, while oil number 1 remained a week or more. (See also the data on penetration in part III of this paper.)

At the expiration of the first twenty-four hours and at certain intervals afterwards, samples were taken from all four plants simultaneously and were treated together, each sample being cut to a distinctive pattern to facilitate its recognition.

After the first 24 hours, samples were taken every three days until it became evident that the withdrawal or disappearance of oil from the tissue of the leaf, even in the case of the lighter oils, was a very lengthy process. The time between samplings was then lengthened to one week.

The disappearance of oil, either from the surface or from within the leaf, was not caused by evaporation, as is generally supposed, except perhaps in a very minor degree. As a matter of fact, the evaporation of oils of high boiling points and low vapor pressures (lubricating oils) is probably a negligible factor at ordinary atmospheric temperatures, especially when enclosed within the intercellular spaces of the leaf tissue. Only those oils with boiling points within or near atmospheric temperature ranges evaporate quickly, *i.e.*, benzine, gasoline, kerosene, etc.

Each set of samples was prepared and mounted both in flat gross mounts and cross-sections, carefully studied under the microscope and compared with preceding lots.

MANNER OF PENETRATION

Samples taken at the expiration of one hour after treatment showed that the cutinized outer integument covering the epidermal cells absorbs oil rapidly. On the upper surface of the leaf the oil accumulates in the depressions between the cells as shown in fig. 2 A. On the lower epidermis it pours through the stomata and runs along the cell walls, frequently coalescing into droplets between the outer walls of the guard cells (fig. 2 B, C). Such droplets disengage themselves and pass into the intercellular spaces of the spongy chlorenchyma. When drops are not formed at this point the oil may run along the outer surface of the epidermal cell wall and gradually spread out between the cells of the spongy chlorenchyma.

At the end of the second hour this process shows further developments. A considerable amount of oil now shows along the outer surface of the

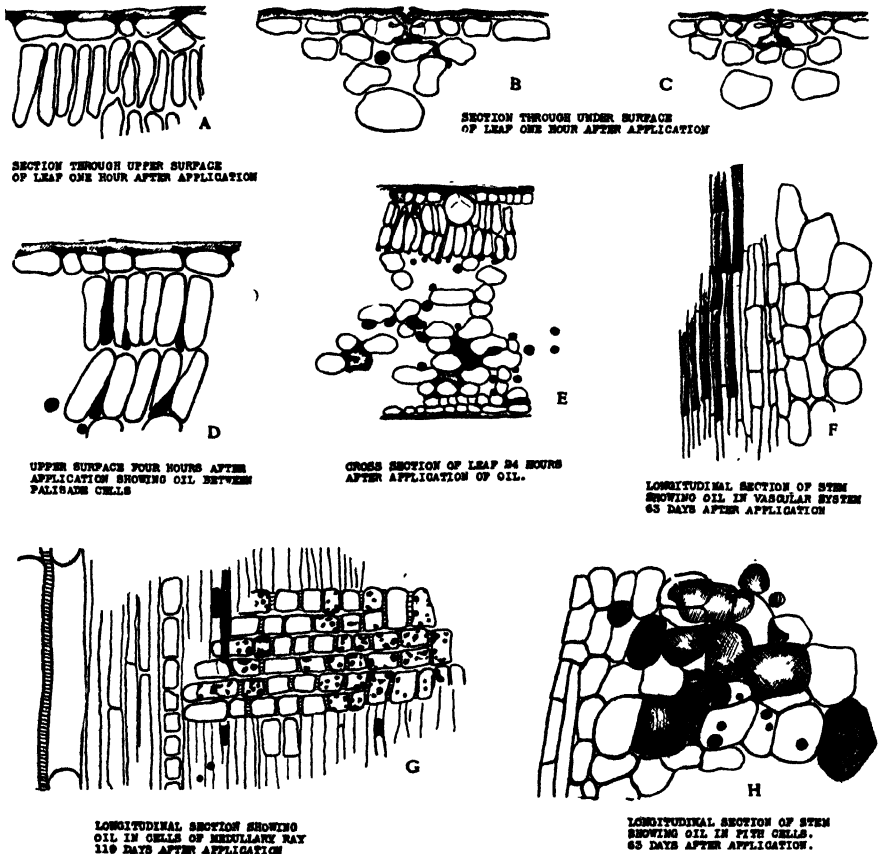


FIG. 2. Internal effects of oil applications to leaf surfaces.

epidermal cells and there are a number of small droplets scattered here and there in the intercellular spaces. Little difference is to be observed on the upper surface except that the oil has penetrated deeper into the depressions between the epidermal cells. By the fourth hour, however, oil begins to appear in the palisade parenchyma (fig. 2 D), apparently having been absorbed by the cell walls and carried thence through the epidermal layer. It is first seen as a minute "thread" squeezed between the long cells, gradually expanding as it descends and finally coalescing in drops at the bottom of the palisade cells and passing into the intercellular spaces of the spongy chlorenchyma. At the end of twenty-four hours oil is entering the leaf from both upper and under surface (fig. 2 E). Quantitatively, however, there is little doubt that the greatest penetration occurs beneath. See part III.

As the penetration of oil from the upper surface had not, apparently, been demonstrated previously, this feature was checked with positive results by treating leaves upon the upper surfaces only.

GENERAL OBSERVATIONS

The oil in the intercellular spaces apparently extracts or dissolves materials from the adjacent cells. In unstained specimens the oil becomes green in color, having evidently dissolved chlorophyll. In stained specimens minute black specks begin to appear in the oil globules at about the third day. These bodies grow larger and coalesce, forming black masses which pass to the periphery of the droplet and become disengaged. At this stage oil can be observed within the cell itself in the form of very minute droplets. It gradually accumulates along the larger veins and passes into the vascular system.

When it became apparent that oil was being translocated steps were taken to ascertain what became of it. Sections were cut from the lateral branch below the treated leaves and also from the main trunk below the point of union with the lateral. Longitudinal and transverse sections of both of these showed large quantities of oil in the phloem (fig. 2 F), the medullary rays (fig. 2 G), and finally in the large storage cells of the pith and old wood fiber of the xylem (fig. 2 H). Some cells became full of oil, others were apparently in process of being filled. Even in those cells already packed with starch grains, oil can distinctly be seen penetrating between them. No oil has been observed in wood of the current season's growth; it is carried across and deposited in the pith parenchyma and the *old* wood fibers. How far down the limb this process extends is not known, but it is probable that most of the oil is deposited in the smaller twigs and limbs and this may account for the great increase in deadwood noted by observers in the field following an application of heavy oil.

Summarizing, the several distinct phases in the elimination of oil from the leaves are seen to be:

- (1) Penetration into the intercellular spaces from the leaf surface.
- (2) Entrance of the oil into the cell.
- (3) Entrance of the oil into the vascular system and translocation down the leaf trace into the stem.
- (4) Transfer from the phloem across the medullary rays.
- (5) Deposit of the oil in the large storage cells of the pith and in the old wood fibers of the xylem.

OBSERVATIONS ON PARTICULAR OILS

Oil number 4.—This was the first one to disappear from the leaves. It is less viscous than oil number 3 although of about the same degree of volatility. It is an unsaturated oil and caused some injury to the leaves, destroying the cells in spots, and causing some of the leaves to drop. The older leaves lost their dark green color and became yellowish, but they regained their color later when the oil disappeared. The last trace of oil was found in the leaf on the 65th day after application. One hundred days after treatment, sections were cut from the limbs below the treated leaves. Both longitudinal and transverse sections showed much oil in the phloem, the medullary rays and the pith and old wood fibers of the xylem.

Oil number 3.—The last trace of this oil was found in the leaf on the 107th day after treatment, 42 days after oil number 4 had disappeared. Oil was still present in the veins of the leaf at this time. One hundred and nineteen days after application and twelve days after the oil had disappeared from the leaf, sections were taken from the stem about seven to eight centimeters below the lowest treated leaf (ten normal untreated leaves occupied this interval). A large quantity of oil was found in the phloem, in the medullary rays and in the pith and old wood fibers of the xylem.

Oil number 2.—The last trace of this oil was found in the leaf 121 days after application. There was still much oil in the veins, and sections from the stem made later showed the same condition as that described above for number 3.

Oil number 1.—This was the most viscous oil tested (106–110 seconds SAYBOLDT). *A large quantity of this oil was still in the leaf 257 days after application.* The translocation of this particular oil is extremely slow, in correlation with its high viscosity. It may, either partially or completely choke certain elements of the vascular system of the leaf for long periods of time. Inasmuch as oil has been found in the leaves of specimens taken in a commercially sprayed grove no less than sixteen months (480 days) after application, it is not improbable that some oil remains in the leaf during most, if not all, of its life period. It was therefore decided to

terminate the test. The stem was sectioned below the treated leaves. As in the previous instances a large quantity of oil was found in the phloem and some in the cells of the pith.

Checks from untreated plots prepared by the same technique were frequently made, both from leaves and stems. Fatty substances, lipoids, resins, essential oils and cutin associated with the leaves and cells were stained but could easily be distinguished from the petroleum by their much darker color.

From the data presented it may be concluded that viscosity is the single factor of greatest importance in determining the mobility of an absorbed oil, and the length of time the oil remains in the leaf and vascular system. The period of persistence is apparently independent of unsaturation provided the tissues are not actually killed. Volatility, as a factor influencing the disappearance of an oil of high boiling point from the intercellular spaces of the leaf, may be disregarded in practice, inasmuch as this study demonstrates that the bulk of this disappearance is due to translocation to other parts of the plant. The lighter oils, being the easiest to move, are the first to disappear.

It appears quite evident that the *accumulation of starch in the leaves, noted in part I of this paper, is due, not to any real stimulation of the plant, but simply to the fact that as the leaf begins to function again, it manufactures carbohydrates which it cannot effectively translocate due to the overloading of the conducting vessels with oil.*

If this condition persists over a protracted period of time it must eventually react unfavorably on the root system, thus weakening the entire tree.

The foregoing data suggest an adequate explanation for the many adverse physiological effects observed in the field following the application of heavy, saturated, white petroleum oil sprays.

Part III

ON SOME FACTORS IN THE PROBLEM OF THE ABSORPTION AND TRANSLOCATION OF SATURATED PETROLEUM OIL IN THE LIVING PLANT

THE INITIAL PENETRATION OF OIL INTO THE LEAF.—It is generally assumed that penetration of oil into the leaf is by way of the stomata. On the whole, this belief seems to be sound. The length of time a surface film remains on a treated leaf is found to vary with the type of oil and the character of the cuticle. Thus a light oil (kerosene for example) applied to a succulent, herbaceous leaf which is supplied with large numbers of stomata disappears from the surface rapidly, the time varying from a few minutes to an hour or so. On leaves of the California Live Oak no free oil (of the kerosene type) persists longer than twenty-four hours. No doubt some of

this disappearance is due to volatility. But with a lubricating oil of high viscosity (100–110) the case is different. Oil applied to a *Malva parviflora* leaf did not disappear entirely until the end of the third day. In the case of the leathery evergreen leaves of the California Live Oak (*Quercus agrifolia*) and California Bay (*Umbellularia californica*), the time is very greatly increased. On the Bay leaves there were traces of free oil sixteen days after application, and oil did not disappear from the Live Oak leaves till some time later. The presence of oil was still visible *inside* the leaves of both Bay and Live Oak seven months after application although in apparently reduced amount. Leaves of these two plants were also treated on their upper surfaces only. The period of persistence of free oil was greatly lengthened, and in both leaves when penetration did occur it took place primarily along the margins and tip of the *underside* where the oil had drained from the upper surface. Some dorsal penetration was observed to occur in the Live Oak leaf where it could not be explained by “under-run” but this did not take place to an appreciable extent until the March following the application—a period of three months.

In order to learn more concerning the rate of this initial penetration, leaves were removed from different plants and immersed in pure oil. The time necessary for complete penetration was recorded. The entrance of oil into the intercellular spaces causes the leaf to become translucent. Complete translucence was taken as the criterion of complete penetration. A representative sample of the data obtained is given in the following table (table IV).

The great difference in susceptibility to oil-penetration by different leaves is clearly illustrated in the table mentioned. Even those leaves most resistant to complete penetration (*e.g.*, Live Oak) show considerable and almost immediate partial penetration. Evergreen leaves are, on the whole, more resistant to penetration than herbaceous leaves, and leaves adapted to xerophytic conditions are most resistant of all.

The effect which viscosity plays in penetration is evident in the tests which follow. The leaves of Cranesbill (*Erodium* sp.) immersed in kerosene show complete penetration in 40 to 60 minutes. In saturated petroleum oil of 110 seconds viscosity, it required 300 to 360 minutes.

The resistance of xerophytic leaves of either the succulent or leathery type (*e.g.*, Stonecrop and Live Oak) is due to imperviousness of the epidermis. Penetration in such leaves is very rapid if an artificial opening is made through the epidermis. The succulent tissues of the Stonecrop absorb oil as so much blotting paper if the epidermis be first stripped off. They seem to resist penetration indefinitely if it be left intact. The variation of the method of penetration into different leaves is shown by the following experiment. A lemon leaf and a Live Oak leaf were taken and the tips sliced

TABLE IV

PENETRATION OF KEROSENE OIL INTO LEAVES OF VARIOUS KINDS. (ISOLATED LEAVES IN PANS OF PURE KEROSENE AT ROOM TEMPERATURE.)

SPECIES OF PLANT	TYPE OF LEAF	NATURE AND DEGREE OF PENETRATION AT END OF 15 MINUTES ¹	TYPE OF PENETRATION	TIME OBSERVED FOR COMPLETE PENETRATION TO OCCUR
<i>Montia</i> sp. (Miner's Lettuce)	Succulent, moisture-loving mesophyte	100 per cent.	Uniform	A few seconds; absorbs oil like "blotting paper"
<i>Sambucus</i> sp. (Elder)	Soft; mesophytic. Veins non-sclerenchymatous ²	About 50 per cent.	Grease-spot ³	1 hour
<i>Coprosma</i> sp. (New Zealand Looking Glass Plant)	Strongly cutinized arid mesophytic. Midrib and large veins sclerenchymatous	25-30 per cent.	Large checkered ⁴	3-4 hours
<i>Rubus</i> sp. (Blackberry)	Soft, hairy, mesophytic. Middle and large veins sclerenchymatous	A few large polygons show partial penetration	Large checkered	10-12 hours
<i>Citrus</i> sp. (Lemon)	Oily, leathery. Non-sclerenchymatously veined	Indefinite blotches show faint partial penetration	Grease-spot	
<i>Umbellularia californica</i> (California Bay tree)	Oily, leathery. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	About 6 days (140-150 hours)
<i>Quercus agrifolia</i> (California Live Oak)	Leathery, non-oily xerophytic. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	40 per cent. complete at end of eighth day (192 hours)
<i>Sedum</i> sp. (Stonecrop)	Xerophytic, succulent (of "cactus type")	None	None	At end of eighth hour there was no indication that penetration had so much as begun. (Not observed further)

¹ Degree of penetration, eye estimates only.² Grease-spot penetration occurs in leaves in which the veins do not form more than a temporary barrier to the lateral diffusion of oil, and in which spread is from a more or less definite center, so that it appears as a circular grease-spot.³ The term *sclerenchymatous* is here used in the sense that such tissues are sufficiently abundant around the veins to completely or very nearly stop lateral oil diffusion across the veins.⁴ Checkered penetration occurs where there is a network of sclerenchymatous veins which inhibit lateral diffusion so that each enclosed polygon of the venous network must be penetrated independently.

off with a razor. The cut leaves were then immersed in oil. The oil rose by capillarity in the lemon leaf and ultimately completely filled the intercellular spaces up to the point of union of the blade with the petiole. In the oak leaf, however, only the mesophyllic sclerenchyma-enclosed polygons which were opened by the razor cut filled with oil. This occurred almost immediately, but there penetration stopped indefinitely.

These observations suffice to illustrate the main features of the phenomena under consideration and to indicate that the factors surrounding oil penetration vary both with respect to the type of oil and the character of the leaf upon which it is applied. The main factor so far as oil itself is concerned is without doubt viscosity.

TISSUE KILLING BY SATURATED PETROLEUM OILS.—When white oils were first used as insecticides, verified evidence as to “burn,” *i.e.*, actual tissue killing, was lacking. It has since been established that over-application of some of these oils does result in killing twigs and even branches of orchard trees. There are indications that true leaf-burn may also occur, but in all cases which have been personally observed this occurs only when the intercellular spaces are completely filled with oil and rendered translucent. This does not ordinarily occur in the field because the amount of oil applied is generally less than the capacity of the intercellular spaces.

Leaves of the California Live Oak and California Bay tree were painted with a saturated petroleum oil of 110 seconds viscosity on December 20, 1926. Five months later, of the 57 Bay leaves treated, 3 had dropped; 2 showed a ten per cent. tip burn, and nearly all the rest showed small marginal areas which had been killed by the oil and had eroded away leaving the leaf margins irregular. Of the 33 leaves observed as a check, one had dropped in that period; the rest were normal. The under surface of the Bay leaf normally possesses a distinct waxy bloom. This was destroyed and had not returned at the end of five months.

The results were similar but more severe in the case of the oak leaves. At the end of the five months observation period, of the 48 leaves treated, 36 had dropped; 2 showed 20 per cent. burn and ten appeared nearly normal. No drop or injury showed on the leaves kept under observation as a check. Leaves treated with ordinary kerosene behaved in all respects as the check lot. The bloom on the under surface of the Bay leaves although dissolved as completely by the kerosene as the heavy oil, had returned within six weeks after the application.

On succulent herbs the effect is still more pronounced. Of 100 “Cranesbill” (*Erodium* sp.) leaflets treated with the saturated viscous oil above noted, 33 were “normal” (still impregnated with oil); 27 burned or yellowed and 40 completely dead 15 days after treatment. At the end of one

month all were dead. On this plant kerosene kills or "burns out" large areas of leaf surface where penetration is greatest. This burning occurs within a day or two after the application. But those areas which are not immediately killed do not subsequently die or show other deleterious effects.

TRANSLOCATION OF OIL IN THE LIVING PLANT.—In order to verify the observations reported in part II concerning the translocation of oil from the leaves to the pith of Citrus plants, leaves of the English Laurel (*Laurocerasus laurocerasus*), a glossy leaved evergreen, were painted with saturated petroleum oil of 106–110 seconds viscosity. Six weeks later a section of the stem well below the treated leaves was removed, the bark shaved off, and the resulting woody cylinder carefully scraped to avoid any possible surface contamination; this was finely sliced with a sharp razor and extracted with CCl_4 . A similar extraction was made of a sample from a normal branch. From the treated branch 11.2 mg. of CCl_4 -soluble material per gram weight of tissue were recovered, while the check yielded just half as much or 5.6 mg. per gram of tissue.

Potato plants were grown and the leaves were treated with the same oil noted above, just prior to the time of tuber formation. After two weeks further growth, the plants were uprooted and the young tubers were washed, sliced and extracted with CCl_4 . The tubers of the treated plant yielded 5.4 mg. of CCl_4 -soluble material per gram while the check yielded 1.8 mg. The test was repeated, the treated plant yielding 18.6 mg. of CCl_4 -soluble material per gram of tuber, the check yielding 7.8 mg. There is a large quantitative discrepancy here which may be explained by the fact that the second lot of material was more finely chopped and was extracted for a longer period of time, but inasmuch as each lot was treated in the same manner as its control, the general conclusion remains unchanged. In addition, the presence of oil exerted a profound effect on tuber formation, those of the treated plant were the size of peas at the time of testing, while those of the check were the size of large marbles, i.e., four or five times as large.

The ability of the plant to absorb and translocate oil was then tested in a third way. Clean river sand was intimately mixed with the same oil noted above in the proportion of 6.8 cc. of oil to 340 grams of sand. Beans were germinated in this substratum and when the plants had reached a height of ten centimeters they were cut off just above the ground line, finely chopped and extracted with CCl_4 . From one lot 8.2 mg. of CCl_4 -soluble material per gram of tissue were obtained and from a second lot 10 mg. The check yielded 3.8 mg. under the same conditions. The test was repeated with substantially the same results; the plant grown in oiled sand yielded 15 mg. of CCl_4 -soluble material per gram of tissue as compared with 5 mg. for the check.

The sand in the preceding experiment did not appear particularly oily and the plants grown therein were apparently normal.

These results demonstrate the reality of cellular penetration of oil and indicate that the plant is capable of translocating oil either from root to leaf or vice versa.

VOLATILITY AS A FACTOR IN THE DISAPPEARANCE OF VISCOUS PETROLEUM OIL

Volatility has been regarded as *one*, if not the *only*, important factor in oil disappearance from a sprayed plant. As late as August, 1928, DE ONG (3) suggests volatility as the primary factor in oil disappearance. This is shown to be in error in the present paper. However, it is certain that even heavy lubricating oils when exposed to air do volatilize to a certain very limited extent. This was tested as follows: Oils were painted in thin films on clean glass plates. One set was exposed to sunlight for a period of eight months under conditions which admitted air but excluded dust. A second set was kept in the dark in the laboratory. Weighings were made from time to time and noted. The results are summarized in table V.

TABLE V

VOLATILITY OR WEIGHT CHANGES IN THIN OIL FILMS ON GLASS, IN FREE AIR
(NOVEMBER, 1927, TO JUNE, 1928)

OIL	Mg. OIL TO AREA OF GLASS	EXPOSURE	PER CENT. CHANGE IN WEIGHT AFTER 2 MONTHS EX- POSURE	PER CENT. CHANGE IN WEIGHT AFTER 8 MONTHS EX- POSURE
Saturated White Western Viscosity 106- 110	10.8 mg.	Sunlight	Loss 43 per cent.	Loss 89 per cent.
	25.5 sq. cm.			
	23.7 mg.	Dark	Loss 14 per cent.	Loss 27 per cent.
	51.6 sq. cm.			
Saturated White Western Viscosity 75	16.6 mg.	Sunlight	Loss 87 per cent.	Loss 91 per cent.
	51.7 sq. cm.			
	17.0 mg.	Dark	Loss 47 per cent.	Loss 82 per cent.
	51.7 sq. cm.			
Saturated White Eastern Viscosity 75-85	15.8 mg.	Sunlight	Loss 12 per cent.	Loss 82 per cent.
	51.8 sq. cm.			
	11.0 mg.	Dark	Gain 2 per cent.	Loss 4 per cent.
	51.2 sq. cm.			

From these data it is evident that volatility even in the case of heavy oil does occur under the conditions of this experiment. It is important to keep in mind however that similar conditions do not obtain when oil is applied to the plant; in the latter case absorption takes place immediately and is

complete in a few days; once within the tissue, the oil is "bottled up." Optical observations in the latter case render it certain that volatility is negligible and does not nearly equal the figures given above. Furthermore, petroleum oils are not constant boiling point mixtures. Evaporation is therefore most rapid at first and involves only the lighter fractions leaving the heavier and less volatile residue behind. Once inside the leaf, oil disappearance is no doubt due primarily to translocation.

Summary and conclusions

1. Experience has shown that saturated petroleum oils, although not toxic to plants in the ordinary sense of the word, are nevertheless the cause of more or less profound and long continued metabolic disturbances in the plant which may ultimately become seriously deleterious.

2. These metabolic disturbances appear to be due to physical rather than chemical handicaps imposed by the intrusion of the oil into the plant tissue.

3. Oil applied as a free cuticular film, either by means of a brush or a quick-breaking emulsion, persists as such, for a period of a few minutes to fifteen or twenty days, depending primarily upon the morphology of the leaf and the viscosity of the oil.

4. The disappearance of the oil as a free surface film is due to its absorption by the leaf. A certain amount of oil is dissipated in gaseous form at this time. This amount is negligible except in the case of non-lubricating oils.

5. The leaves of plants which are adapted to xerophytic or arid conditions are much more resistant to this initial penetration than softer mesophytic or succulent leaves.

6. The resistance offered to this initial penetration is mostly epidermal, as shown by the fact that penetration in a xerophytic leaf may be extremely rapid once ingress to the interior is obtained. The same factors which protect such leaves from excessive water loss appear to be likewise effective as a protection against the ingress of oil. Penetration is always most rapid in areas rich in stomata but is not necessarily confined thereto. In citrus at least, penetration may occur by seepage between the epidermal cells of the upper (stomata-free) surface of the leaf.

7. Oil penetration is not usually uniform over an entire leaf; certain focal points are first penetrated and the oil tends to spread peripherally from those points. In certain leaves this early peripheral spread is checked more or less completely by the network of veins. Penetration in a leaf of this sort involves the independent absorption by each of the vein-bound polygons of mesophyll.

8. Beginning within a few days after entrance into the intercellular spaces of citrus and extending over a period of many months in the case of

a saturated petroleum oil of 106 seconds viscosity, the oil is taken into the vascular system of the plant and translocated to the storage tissues (pith and xylem parenchyma).

9. In Citrus plants the absorption of oil has been observed directly by microscopical means in the phloem of the leaf traces and stem; in the medullary rays of the stem, and in the pith and xylem parenchyma and sclerenchyma of the stem. No oil has been observed in xylem tissues of the current year's growth.

10. During the period of oil penetration and initial translocation, transpiration is sharply decreased and respiration enormously increased. Photosynthesis becomes temporarily inoperative. This may be due in part to the effects of the oil on the chloroplasts. Intercellular oil is shortly turned green by the extracted chlorophyll.

11. Recovery begins quickest in the case of light oils and is indicated by the return of transpiration and respiration rates towards normal and by the accumulation of carbohydrates in the leaves in abnormally large amounts. This latter phenomenon is correlated with the fact that the conducting vessels (phloem primarily) are still taxed to capacity with the oil and hence cannot adequately handle the carbohydrates now beginning to be produced in excess of the needs of the leaves.

12. The increase of carbohydrates in the leaves of Citrus trees recovering from an oil treatment is therefore in all probability a pathological phenomenon and not an indication of more vigorous synthetic metabolism or "stimulation" as has been maintained.

13. In addition to visual methods (microscopic) the fact of oil accumulation in pith and xylem has been verified by another means. Storage tissues of branches or plants whose leaves had been painted with oil were extracted by means of carbon tetrachloride and the oily materials in such tissues were found to be far in excess of the amounts in similar tissues of untreated plants.

14. It was also found that the plant is capable of transporting oils from the roots upward to the leaves. The leaves of young beans and peas grown in oil-treated sand show a much higher content of oily material than plants grown in normal soils.

15. The intracellular absorption of oils both from the intercellular spaces of the leaf and from the soil indicates the passage of oil molecules through the cell membrane into the cytoplasm of the cell.

16. Microscopic observation of all stages in the translocation of oil was rendered possible by devising a special staining technique which is described in the text.

17. Experiments with oil films on glass plates indicate that volatility is a real factor in the disappearance of even viscous oils in *free air*. In

practise however, due to the enclosure of the oil in the intercellular spaces, it is unquestionably negligible in comparison with translocation.

18. From the effects summarized in the preceding paragraphs the conclusion seems evident that *heavy* white oils (of a viscosity exceeding 60 seconds SAYBOLDT) must be used sparingly and with a great degree of caution, if, in the future, serious ultimate injury is to be avoided.

LITERATURE CITED

1. KNIGHT, HUGH. A micro-technique for observing oil penetration in citrus leaves after spraying. *Science N.S.* **68**: 572. 1928.
2. DE ONG, E. R., KNIGHT, HUGH, and CHAMBERLIN, JOSEPH C. A preliminary study of petroleum oil as an insecticide for citrus trees. *Hilgardia* **2**: 351-383. 1927.
3. ———. Progress report on the use of petroleum oil as an insecticidal spray. *Jour. Econ. Ent.* **21**: 525-529. 1928.
4. PROESCHER, F. Oil red O pyridin, a rapid fat stain. *Stain Techn.* **2**: 60-61. 1927.
5. QUAYLE, H. J. Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. *Jour. Econ. Ent.* **15**: 400-404. 1922.
6. WOGLUM, R. S., LA FOLLETTE, J. R., and LANDON, W. E. Handbook of citrus insect control for 1928. Calif. Fruit Growers' Exchange. Pp. 3-36. 1928.

OPENING OF STOMATA IN DIFFERENT RANGES OF WAVE LENGTHS OF LIGHT¹

J. D. SAYRE

(WITH FOUR FIGURES)

The relations existing between the phenomenon of stomatal behavior and the factors influencing this have been widely studied and discussed. There is a great diversity of opinion concerning the significance of many of these factors in regulating stomatal response. This is particularly true for light since this factor may vary greatly in intensity, duration, and quality, depending on the time of year, the degree of cloudiness, the clearness of the atmosphere, altitude, etc.

The author, in a recent paper (8) dealing with a study of the stomatal physiology of patience dock, *Rumex patientia* L., included a discussion of the phenomenon accompanying the opening and closing of the stomata of this plant. The variation in the size of the stomatal pore for day and night periods is relatively large in this case.

Historical

Not much work has been reported on the effect of light of various regions of the spectrum on stomata. KOHL (6) reported that the rays between B and C and the rays near F were alone effective. FRANCIS DARWIN (2) agreed with KOHL as far as the red end of the spectrum was concerned but did not draw definite conclusions about the blue end. LLOYD (7) reported normal behavior of the stomata in light between 540 m μ and 700 m μ at the red end and also between 420 m μ and 480 m μ at the blue end of the spectrum. No measure of the intensity of the various regions of the spectrum was given by these investigators. Nor was it stated whether that part of the red beyond 700 m μ was tried.

Methods

Daylight was used as the source of light in these experiments. The plants used, patience dock, were grown out-of-doors. They were covered with tin boxes which were ventilated and light tight. Four ray filters, six and a half inches square, were fitted in the top of each box, the box being sunk several inches in the soil around the plant. Observations of the stomata were made with the leaf in position on the plant. The glass plates were placed over the plants in the evening and observations were made at short intervals during the following day.

¹ Paper from the Department of Botany, The Ohio State University, no. 235.

Two series of ray filters were used. The first series, G38L, G38H, G34, and G24², absorb successive portions of the visible spectrum, beginning at the red and extending towards the blue end. The limits of their transmission are shown in figure 1; the shaded areas represent the absorbed regions and the unshaded areas the transmitted regions. This diagram does not

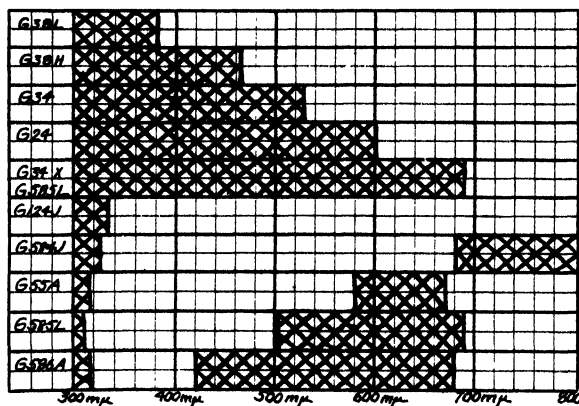


FIG. 1. Limits of transmission of the ray filters used in this work. Shaded areas, absorbed regions; unshaded areas, transmitted regions.

give a true picture of the transmission of all these glass plates. For instances, G55A transmits light to 580 mμ but the percentage transmission between 520 mμ and 580 mμ is very low. For an accurate comparison of the transmission values of these glass plates the Bureau of Standards publications (1, 3, 4) should be consulted. The values are also shown in a previous article by SAYRE (9), in which the curves of transmission for an equal energy spectrum of all these glass plates are given.

The relative distribution of daylight received upon a horizontal surface was obtained from KIMBALL (5). These data which are shown in figures 2, 3 and 4 are the mean values for the different hour angles of the sun, obtained by averaging his values. They are for latitude N 41° and for a cloudless day, September 21.

Kimball's data extend only to 396 mμ in the ultra-violet. Since hardly any light shorter than 300 mμ reaches the earth from the sun, the curve was extended from 396 mμ to 300 mμ as a dotted line. Likewise, in the red end, the last measurements were given at 776 mμ. But as ABBOT and others have shown that the energy from the sun extends far into the infra-red, the curve at this end has been extended as a dotted line to 800

² Trade names or numbers of the glass plates used, Corning Glass Works, Corning, N. Y.

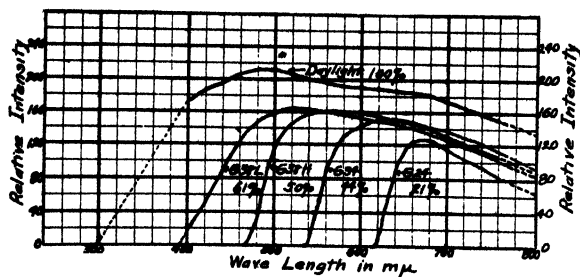


FIG. 2. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by the glass plates: series I.

$m\mu$ on the same slope as from 700 $m\mu$ to 766 $m\mu$. This makes it possible to compare the energy values in this region under the glass plates.

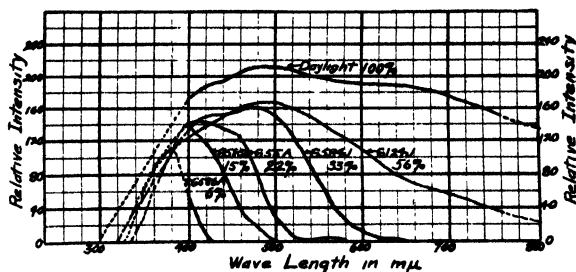


FIG. 3. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by the glass plates: series II.

The relative transmission of daylight by the various glass plates was obtained by multiplying their transmission values for an equal energy spectrum by the relative intensities of daylight at various wave-lengths. The percentage transmission, figures 2 and 3, was calculated from the areas under the curves as compared with the curve of daylight.

No instruments were available for measuring the actual intensities of light under the various glass plates, so this method of calculation had to be used. It is recognized that this method may not be very accurate but it is believed that the results obtained allow certain conclusions to be made.

Results

The results of observations made on clear days are considered here, although the results on partly cloudy days did not differ materially from these. Observations of the stomata under the first series of plates, the transmission data of which are given in figure 2, showed that the stomata opened and closed normally. Observations under the second series, transmission data in figure 3, showed the same results, with the exception of

G586A. These observations would indicate that all regions of the spectrum which are not absorbed by these glass plates are equally effective in bringing about the opening and closing of stomata.

Normal opening of the stomata did not occur under G586A, but since the intensity under this plate is very low, it cannot be determined from these results whether intensity or wave-length was the limiting factor. Until more convenient methods of measuring intensities in this region are available and a better source of ultra-violet light is available this part of the problem must be left unsolved.

It is possible by means of a combination of G585L and G34 to make another division of the spectrum at the red end. G585L has a second transmission band in the red. When G34 is placed over it the whole blue end is absorbed and the band in the red from about 690 $m\mu$ to far beyond the visible spectrum is left unaffected. The intensity under this combination is rather low because two plates are used and only a limited region of the spectrum is involved. Many observations made under this combination showed that there was no opening of the stomata in this region. They behaved just as in complete darkness. Since the intensity was low it was necessary to prove that this failure to open was not due to lower intensity but to specific wave-length of light. This was attempted by observations of the stomata on a cloudless day. Comparisons were made with similar plants placed under 2-G24 plates, G34 + G585L, in complete darkness, and in full daylight.

Figure 4 shows the relative intensity of the daylight received on a horizontal surface and the relative transmission of 2-G24 and G34 + G585L. These data were obtained in a manner similar to that described for the other glass plates. Table I shows the results of observations on a cloudless

TABLE I

RELATIVE TRANSMISSION OF THE GLASS PLATES AND THE WIDTH OF THE STOMATAL PORE OF PATIENCE DOCK

COMBINATIONS OF GLASS PLATES	LIMITS OF TRANSMISSION	TRANSMIS- SION OF DAYLIGHT 300-800 $m\mu$	WIDTH OF THE STOMATAL PORE			
			8:30 A. M.	10:30 A. M.	1:00 P. M.	3:00 P. M.
	$m\mu$	<i>per cent.</i>	μ	μ	μ	μ
No cover	Full daylight	100	10-12	16-18	0-3 ¹	0 ¹
Open box	" "	100	10-12	16-18	0-3 ¹	0 ¹
1-G24	601-Infra red	21	6-8	12-14	18-20	18-20
2-G24	601- " "	12	6-8	12-14	18-20	18-20
G34 + G585L	690- " "	10	1-2	1-2	0	0
Closed	Darkness	0	1-2	0-1	0	0

¹ Leaves badly wilted.

day. The average widths of the stomatal pores are given together with the limits and percentages of transmission of the various glass plates, and suitable checks.

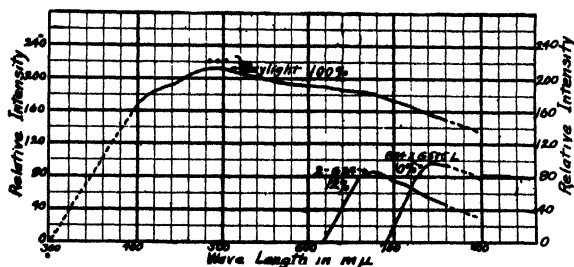


FIG. 4. Relative distribution of daylight received upon a horizontal surface; and the relative and percentage transmission of daylight by 2-G24 and G34 + G585L glass plates.

There was a very slight opening of the stomata under G34 + G585L in the forenoon. Similar behavior was shown in complete darkness. This is a rhythm of the opening of the stomata. It has been reported by several other workers in discussing the behavior of stomata. Some question the existence of such a rhythmic movement, but it can be easily observed and measured in patience dock. It occurs when plants are placed in a dark box after sundown and left there the next day. The stomata remained open under all the other glass plates. Without glass plates they opened normally but closed in the afternoon when the plants wilted badly. Since the intensity under 2-G24 and G34 + G585L was practically the same, the only difference being in the region of the spectrum concerned, we must conclude that wave-lengths of light longer than 680 mμ are not effective in opening of stomata.

In a previous paper (9) it was shown that no formation of chlorophyll as indicated by the greening of seedlings occurred in light of wave-lengths longer than 690 mμ. No phototropic response by plants occur in this region of the spectrum, the limit for this being at 526 mμ. Starch accumulation does not occur beyond 690 mμ. This was shown by placing starch-free *Coleus* plants under the glass plates G34 + G585L, and testing them for starch at frequent intervals. The results of this latest investigation show that stomata do not open in light of wave lengths longer than 690 mμ. The conclusion from all of these facts is that the limit of the red end of the spectrum in its effect on plants is at 690 mμ. Wave-lengths of light longer than 690 mμ are probably absorbed by the plant but they apparently produce no chemical or physiological effects. They would be changed to heat energy and raise the temperature of the plant or be lost by radiation.

Conclusions

1. The stomata of *Rumex patientia* do not open in light of wave lengths longer than 690 m μ .
2. The other regions of the visible spectrum (except the violet and ultra-violet) seem to be equally effective.
3. The limit of the effectiveness of the blue end of the spectrum could not be determined from the results of this experiment.
4. It is suggested that red light beyond 690 m μ does not affect plants except as it may be absorbed and changes to heat energy.

OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO.

LITERATURE CITED

1. COBLENTZ, W. W., and EMERSON, W. B. Glasses for protecting the eyes from injurious radiations. Bur. Standards Technologic Papers, no. 93. (3rd ed.) 1919.
2. DARWIN, FRANCIS. Observations on stomata. Phil. Trans. Roy. Soc. Lond. B. **190**: 531-621. 1898.
3. GIBSON, K. S., and McNICHOLAS, H. G. The ultra-violet and visible transmission of eye-protective glasses. Bur. Standards Technologic Paper, no. 119. 1919.
4. TYNDALL, E. P. T., and McNICHOLAS, H. G. The ultra-violet and visible transmission of various colored glasses. Bur. Standards Technologic Papers, no. 148. 1920.
5. KIMBALL, HERBERT, H. Energy distribution in the visible spectrum of sunlight and skylight. Monthly Weather Rev. **53**: 112-115. 1925.
6. KOHL, F. G. Über Assimilationsenergie und Spaltöffnungsmechanik. Bot. Centralbl. **64**: 109-110. 1895.
7. LLOYD, F. E. The physiology of stomata. Carnegie Inst. Washington Pub. no. 82. 1908.
8. SAYRE, J. D. Physiology of stomata of *Rumex patientia*. Ohio Jour. Sci. **26**: 233-266. 1926.
9. ————. The development of chlorophyll in seedlings in different ranges of wave-lengths of light. Plant Physiol. **3**: 71-77. 1928.

MECHANISM OF CELL WALL FORMATION

O. L. SPONSLER

(WITH THREE FIGURES)

As an outgrowth of x-ray studies on the molecular structure of cellulose walls of plant fibers (12, 13, 14), several steps in the process by which the cell wall grows in thickness, seem to have become evident. They are associated with the regularity of molecular arrangement in the wall and with the forces which produce that regularity of structure. As the x-ray data, during the progress of the investigations, made it more and more certain that the structural units are glucose residues, and that they are arranged with the regularity of the molecules in a crystal, the concept became clearer and more convincing that the mechanism of cell wall growth in thickness was associated intimately with the forces which are active in causing that regular arrangement of the residues, that is, with forces similar to those which produce crystallization.

The mechanism which is described in the following pages is concerned with only one phase of cell wall growth; that is, with the increase in thickness of the cell wall. While it may be associated also with the increase in surface area as the cell becomes enlarged and even with the formation of the original wall, those two phases are not included in the present discussion. The problem then becomes restricted to a consideration of the mechanism by which new cell wall material is deposited upon older previously formed cell wall.

Layers in cell wall

Where fiber-shaped cells have been studied microscopically (1, 2, 11, 15) the wall has been found to consist of many very thin layers (1, 2, 11), which seem to indicate a periodicity in the growth in thickness. Each layer is considered as a deposition of new cell wall material upon the surface of an older layer, and while each thin layer may perhaps have two surfaces available for deposition of new material, we are considering here only the innermost layer of the wall and the surface which is in direct contact with the cytoplasm of the cell; that is, the inner surface of the cell wall. Measurements of these layers show that their thickness is equal to several hundred or perhaps several thousand glucose molecules (12). The x-ray studies indicate that each of these thin layers of microscopic visibility is made up of still thinner layers which are invisible and which are only one glucose residue in thickness (12). The inner surface of the cell wall would be, then, one side of such a layer of glucose residues.

Relation of glucose to cellulose

That the newly deposited material is composed of glucose residues is inferred from the works of various investigators. The new layers have been shown repeatedly through microtechnical work to be composed of cellulose, and cellulose has been shown, through the work of chemists (7, 4) to be composed of glucose residues. The residue is a $C_6H_{10}O_5$ type of carbohydrate, an anhydrous form of glucose; but concerning the nature of the material from which the residue is deposited, that is, the material immediately preceding its appearance as cellulose, there is very little discussion in the literature. No experimental evidence is available to show that the anhydrous glucose units of the cell wall are derived directly from glucose molecules. The assumption of its glucose origin is made from the reverse of that reaction; that is, since cellulose may be converted into glucose, the assumption is that glucose in the cell is converted into cellulose. The former is a common laboratory hydrolysis experiment; the latter, glucose to cellulose, a condensation reaction in which water is split off, has never been accomplished *in vitro*. It is not known then whether the glucose molecule, originating through photosynthesis, becomes transformed first to some component part of the protoplasm and then re-transformed into the carbohydrate, cellulose, or whether it is transformed directly into cellulose.

In developing the mechanism of cell wall growth, we are accepting two conclusions which seem to be reasonable: first, that the deposition of new material is made at the interface between the cytoplasm and the wall; and secondly, that glucose is transformed directly into cellulose. While the mechanism is more readily described by accepting these two concepts they might vary considerably without invalidating the mechanism proposed.

Since the process involves a transformation of glucose molecules at the surface of the cell wall into cellulose molecules which become component parts of the wall structure, a detailed picture of the molecular situation at the interface is necessary.

Molecular structure of cell wall

Interpretation of x-ray data shows that the cell wall of plant fibers is constructed of units or building blocks of molecular size which have an orderly arrangement in three dimensions (6, 10, 5, 12). The unit of structure proves to be an anhydrous residue of β d-glucose (13, 9, 7). The empirical formula for the glucose molecule is $C_6H_{12}O_6$; for the anhydrous residue, $C_6H_{10}O_5$. Spatially these molecules or groups of atoms are three dimensional structures in which the atoms have definite positions allocated to them (13, 9). The distances between the atoms, and their location with reference to one another, are known with sufficient definiteness from x-ray

data (3) and from chemical experimental data (4) to allow a fairly accurate model of the molecule to be constructed. Fig. 2 is a projection of such a model of a β d-glucose molecule (13, 9). The carbon atoms are numbered 1 to 6, and the oxygen atoms are indicated by solid black circles. The positions of only two of the 12 hydrogen atoms are shown, as a matter of convenience. These two are represented by the smaller circles attached to the oxygens, forming the OH groups which in turn are attached to the carbons numbered 1 and 4. This glucose molecule becomes an anhydrous residue when the OH is removed from C₄, and the H from C₁.

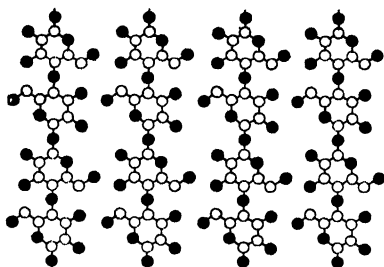


FIG. 1. View of inner surface of cell wall showing four chain molecules. Only four glucose residues of each chain are shown. Solid black circles represent oxygen atoms; light circles, carbon atoms; hydrogen atoms not shown.

In the wall of the fiber each anhydrous residue has a very definite position with reference to the neighboring residues such that the spacing between them in a given direction is uniform. That may be brought out more clearly by referring to fig. 1, where sixteen residues are shown, drawn practically to scale. The distances from center to center of the residues are proportional to the spacings determined from x-ray work with fibers, and their relative positions also are in accord with the x-ray data. They represent, however, a three dimensional structure, and a more truthful picture is obtained when we think of the odd numbered carbons as being raised above the page and the even numbered as being in the plane of the paper. Alternate oxygens are to be considered also as raised above the plane of the paper.

The picture of the structure as given in fig. 1 represents a surface view of the cell wall, such as a minute observer might see, whose senses were attuned to atomic dimensions, if he were inside the fiber looking directly at the inner face of the cell wall. The units or residues would appear to be attached to one another, lengthwise of the fiber, through an oxygen atom acting as a bridge between each two residues. The effect would be that of long chains with the residues acting as links of the chain. The chains themselves, however, because of the greater distance which separates them from one another, would seem to be suspended in space. Fig. 1, then, would

represent a section of the inner surface of the wall showing only parts of four chains, each part containing only four residues.

Inner surface of cell wall

On looking through that surface layer of chains, other similar layers would be seen, all of them parallel to the surface and separated from one another by uniform distances. The distances separating the layers would be nearly the same as those separating the chains. We are especially interested in the layer which forms the inner surface of the cell wall, because it is upon that layer that the new material is deposited. New residues which may be laid down on this surface become arranged in the same orderly manner as the previously deposited residues; that is, they take up positions which result in an extension of the lattice structure towards the interior of the cell.

Inner surface similar to crystal face

The regularity in arrangement of the structural units of the cell wall as revealed by x-ray studies is essentially the regularity of crystal structure; that is, the residues in the cell wall are directly comparable to the molecules in a crystal as, for example, the molecules of sugar in a crystal of sugar. The residues, like the sugar molecules, are not only spaced in an orderly manner but are also oriented in a definite way with respect to one another. It would seem then that forces are involved in locating and orienting the residues in the cell wall, which are comparable to those acting in a similar way on the sugar molecules during the formation of a sugar crystal.

The general conception concerning these forces is that they are due to the electrical conditions occurring in the component atoms, which set up electrostatic force fields around the molecule; and that these force fields are responsible for the cohesion between the molecules in the crystal (8). In some way these force fields produce the orientation of the molecules when they are being deposited on the crystal face; and they also determine the location of the molecule. The position which the molecule is forced to take is one of minimum potential energy which is also the position of a unit of the crystal lattice.

Since the glucose residues act as lattice units of the cell wall, one is led to think that the force fields which determine their position and orientation are similar to those acting when molecules are deposited on a crystal face. The inner surface of the cell wall then may be considered as similar to a crystal face as regards the distribution of forces which are involved in the deposition of new lattice units.

Glucose molecule and its anhydrous residue

There is, however, this difference, that the sugar molecule has very probably the same structure in solution as it has after it is deposited as a unit of the sugar crystal; while the glucose molecule in the cytoplasm is slightly different in its structure from the residue which acts as the unit in the cell wall. As a matter of fact there is a great probability that the β d-glucose molecule is only very slightly different from its anhydrous residue, the difference between them being the loss of one OH group and one H atom from carbons number 1 and 4, respectively, or vice versa. That may be visualized readily from fig. 2. It may be that the possibility of the formation of cellulose is due to that particular difference and similarity. There seems to be no fundamental reason for thinking that the component atoms of the molecule and of the residue differ in their general spatial relations to one another, although that has not been definitely proved.

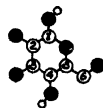


Fig. 2. A single glucose molecule. The carbon atoms are designated by number. Only two hydrogen atoms are shown.

Transformation of glucose to cellulose

When we accept the general similarity of the two, and assume that the glucose molecule could be deposited on the surface of the cell wall as though it were a residue, a very interesting state of affairs is brought out. The molecule would probably take a position of minimum potential energy,



Fig. 3. Two glucose molecules placed as they are oriented in the cellulose chain, to show the position of the OH's at time of condensation. Smaller circles represent hydrogen atoms.

which would be the position of a unit of the lattice; also it would be oriented nearly, if not exactly, as a residue would be if in that place. That may become clearer by imagining a glucose molecule (fig. 2) as finding the proper position over a surface such as represented by fig. 1, and thinking of the molecule as the starting point of a new layer. A second molecule may be expected to fit into an adjacent position. This could be represented by substituting fig. 3 for fig. 2. The force fields, or we may say the cohesion, should hold these two molecules practically as firmly to the surface layer as would be the case if they were anhydrous residues. When the molecules of fig. 3 are forced into the proper orientation by forces of the surface layer, the two OH's attached to the numbers

1 carbons are brought into close proximity. That is decidedly an advantageous spatial relation for a condensation reaction to take place. The result of such a reaction is the formation of a water molecule, $\text{OH} + \text{H} = \text{H}_2\text{O}$; and at the same time, the formation of an oxygen bridge between the two glucose

residues in which the remaining O is used. The paragraph may be summed up by saying that the force fields, resulting from the regular distribution of the glucose residues in the wall, have made the conditions favorable for a condensation reaction, such as the organic chemist usually expects when two OH groups are brought close together.

Relation of lattice to long chain formation

The conditions are made favorable, however, for the reaction to take place in one direction only. It may be seen in fig. 1 that the distance from center to center of the residues is too great laterally for condensation to occur between adjacent units of two chains; while in a lengthwise direction of the chains the distance is extremely favorable for the reaction. That would allow an indefinite number of glucose molecules to be brought into position in which the condensations would be made probable; and the formation of a long straight chain of residues linked by oxygen bridges might readily follow.

Relation of glucose structure to chain molecule

The structure of the β d-glucose molecule also favors the straight chain formation. Evidence from both chemical (4) and x-ray work (13, 9) shows that the oxygen bridges are associated with carbons number 1 and 4. These two carbon atoms are found to be located on opposite sides of the glucose molecule model. That location places them in positions in the molecule which are decidedly favorable for the condensation reaction, and in positions which permit of a straight chain formation.

Evidence of a third factor

Although the molecular and atomic arrangements and dimensions all tend towards the formation of cellulose chain molecules, the mechanism described above cannot account for all of the observed facts. The mechanism mentioned requires only two factors, (1) the cellulose surface and (2) the presence of glucose molecules. It is well known, however, that glucose may be present in a cell and no growth take place in the thickness of the wall. A third factor, then, must be involved in the growth. It is also well known that no growth in thickness of the cell wall occurs after the protoplasm has become inactive, although glucose may be present. The third factor, then, must be associated with some reaction which can occur only when living protoplasm is present. Another well known fact, that the wall may grow on one side of the cell and not on another side, indicate that the third factor is localized in the cell. And since chunks of cellulose do not occur at random throughout the protoplasmic mass inside the cell it would seem that the third factor is localized only at the interface between the cytoplasm and the wall.

Suggestions as to the nature of that third factor are rather meager. Speculation suggests many lines of approach. Careful studies of the conditions inside of the cell at the time the cell wall is being thickened, studies with a view point to determining the concentration of glucoses, and the forms in which they occur, the rate of respiration, the water content of the cytoplasm, the presence of dehydrating bodies, etc., may throw some light upon the remaining parts of the mechanism.

The use of the expression "third factor" or "third body" is, of course, merely symbolical of whatever process or processes must be carried on to complete the condensation reaction which was made possible by the surface forces of the inner face of the cell wall.

Summary

When considering the growth of a cell wall, three stages in the process may in general be recognized: (1) the formation of the original or first layers of cellulose; (2) the surface area growth of the wall as the cell becomes larger; and (3) the increase in thickness of the wall. The third stage only is under consideration in this paper. An attempt has been made here to show that inherent in the molecular structure of the wall are forces which must share very largely in the process of building up the wall itself in thickness at least.

An outstanding feature in the mechanism of thickness growth, it may be pointed out, is the part played by a *surface of regularly spaced molecular units*. The necessity of a surface of some kind on which cell wall material may be deposited is in accord with microscopic studies; but concerning the molecular structure of the surface, only one kind of foundation surface, that of the cellulose wall itself, has so far been investigated. The preceding discussions, then, refer only to cellulose surfaces in which the regularity of arrangement of the structural units, β d-glucose residues, suggests a comparison with crystal surfaces in which large molecules act as structural units. From that comparison one is led to conclude that the forces of crystallization form a part of the mechanism by which glucose molecules become converted into cellulose. Upon following that line of thought farther, it was found that on account of the particular structure of the glucose molecule an opportunity was provided for a condensation reaction which would produce long straight chains of residues, or cellulose molecules.

It was pointed out that crystallization forces alone could not account completely for the transformation of glucose to cellulose; that a third factor which seemed to be associated with living protoplasm was necessary. No discussion of the nature of that third factor is given.

LITERATURE CITED

1. ALDABA, V. C. The structure and development of the cell wall in plants. I. Bast fibers of *Boehmeria* and *Linum*. Amer. Jour. Bot. **14**: 16-24. 1927.
2. BALLS, W. L. The development and properties of raw cotton. p. 73. London. 1915.
3. BRAGG, SIR W. H. and W. L. X-rays and crystal structure. London. 1924.
4. CHARLTON, W., HAWORTH, WALTER NORMAN, and PEAT, STANLEY. A revision of the structural formula of glucose. Jour. Chem. Soc. London **129**: 89-101. 1926.
5. HERZOG, R. O. Röntgenspektrographische Untersuchungen der Cellulose. Cellulosechemie **2**: 101-102. 1921.
6. ————— and JANCKE, WILLI. Röntgenspektrographische Beobachtungen an Cellulose. Zeitschr. Physik. **3**: 196-198. 1920.
7. IRVINE, JAMES COLQUHOUN. The constitution of polysaccharides. Chem. Reviews **1**: 41-71. 1924.
8. JOFFÉ, A. F. The physics of crystals. New York. 1928.
9. MEYER, KURT H., and MARK, H. Über den Bau des krystallisierten Anteils der Cellulose. Ber. deutsch. chem. Ges. **61**: 593-614. 1928.
10. POLANYI, M. Das Röntgen-Faserdiagramm. Zeitschr. Physik. **7**: 149-180. 1921.
11. RITTER, GEORGE J. Composition and structure of the cell wall of wood. Jour. Ind. Eng. Chem. **20**: 941-945. 1928.
12. SPONSLER, O. L. X-ray diffraction patterns from plant fibers. Jour. Gen. Physiol. **9**: 221-233. 1925-1926. Also, Molecular structure of plant fibers determined by X-rays. Jour. Gen. Physiol. **9**: 677-695. 1925-1926.
13. ————— and DORE, W. H. The structure of ramie cellulose as derived from X-ray data. Colloid Symposium Monograph **4**: 174-202. 1926.
14. —————, —————. The structure of mercerized cellulose. I. The space lattice of mercerized ramie cellulose as developed from X-ray data. Jour. Amer. Chem. Soc. **50**: 1940-1950. 1928.
15. VAN WISSELINGH, C. Die Zellmembran. Berlin. 1924.

THE PURE PIGMENTS, CAROTIN AND XANTHOPHYLL, AND THE TSWETT ADSORPTION METHOD¹

F. M. SCHERTZ

Introduction

Ever since the yellow plastid pigments have been investigated in plant organs, especially in leaves, there has been considerable controversy regarding the nature and the number of the pigments present. STOKES supposed that there were two and SORBY three xanthophylls. BORODIN observed that many crystallizable yellow substances, whose solubilities differed, were present in leaves. He recognized two groups, the carotins, to which belong crystals easily soluble in benzene and difficultly soluble in alcohol, and a second group the representatives of which dissolve very slightly in benzene and easily in alcohol. ARNAUD believed that only a single yellow pigment was present and that this was carotin. MONTEVERDE, TSCHIRCH, TSWETT and SCHUNCK confirmed and enlarged upon these observations. Later WILLSTÄTTER and MIEG isolated a representative of each of the groups as designated by BORODIN. The large yield of carotinoids obtained by WILLSTÄTTER and his coworkers makes it seem highly improbable that other yellow plastid pigments are present (10). A very complete review of the literature is given in the monograph of WILLSTÄTTER and STOLL in chapter XII.

TSWETT by means of his chromatographic adsorption apparatus has attempted to further resolve the yellow pigments. For example, by filtering a carbon disulphide solution of the leaf pigments through a column of calcium carbonate, carotin passes through without being adsorbed. In addition to the green pigmented zones are four zones of yellow pigments, which TSWETT distinguished as α , α^I , α^{II} , and β . In the spectrum they show small relative differences in the position of their absorption bands, and as yet these pigments have not been crystallized or isolated in the pure state. TSWETT holds the view that the xanthophyll of WILLSTÄTTER and his coworkers is a mixture of two or three isomorphous xanthophylls while WILLSTÄTTER has suggested that it is possible that the original pigments have been changed by oxidation during the process of separation. It is the purpose of this paper to support the contention of WILLSTÄTTER.

¹ Soil Fertility Investigations, United States Department of Agriculture, Washington, D. C.

Inconsistencies in recent literature

Results described in recent literature show that the method of TSWETT has been employed, and in a paper on xanthophyll (5) certain inconsistencies reported in the literature have been noted by the writer. The inconsistencies referred to are to be found in a paper by GILL (1) and others in a paper by PALMER and ECKLES (3).

GILL states that "in certain cases none of the pigments is extracted from a petroleum ether solution of the pigments by 80-90 per cent. alcohol, while large amounts of the pigments are adsorbed by calcium carbonate." Since the pigment is not extracted from petroleum ether it would be carotin; and since it is adsorbed by calcium carbonate it would be xanthophyll. But surely it cannot be both pigments at the same time.

PALMER and ECKLES report that the "xanthophyll-like constituents of yellow corn were not adsorbed by calcium carbonate from a carbon disulphide solution and on the other hand the pigments could be completely extracted from a petroleum ether solution by 80 per cent. alcohol."

No attempt was made by these authors to explain their results and in fact nothing was said in their work regarding the peculiar behavior of the yellow pigments, with which they were dealing. It would appear then that either the method of fractionation as described by WILLSTÄTTER must be faulty or that TSWETT's adsorption method does not separate xanthophyll from carotin.

Before turning our attention to an explanation of the inconsistencies mentioned above it will perhaps be of interest to have briefly summarized some of the statements made by various writers concerning the different xanthophylls which have been described.

THE XANTHOPHYLLS OF KOHL

KOHL (2) described two xanthophylls. The xanthophyll as described by SCHUNCK, KOHL called α xanthophyll, which has four absorption bands beyond the Fraunhofer line F. This xanthophyll was not obtained in crystalline form, yet it has many of the properties of the xanthophyll described by WILLSTÄTTER and may be regarded as representative of the xanthophyll which we know today.

The xanthophyll described by TSCHIRCH, KOHL has designated as β xanthophyll. This xanthophyll KOHL states is present in all types of leaves, green, yellow and autumnal; and a solution of it possesses only end absorption. β xanthophyll was present mostly in the cell sap and could be extracted by boiling water, forming a dark yellow, yellow, or yellow brown solution. When ammonia was added the aqueous solution darkened and with alkali it became orange colored. Petroleum ether would not extract

any of this yellow pigment from an alkaline solution. If the leaves were extracted with alcohol after the water extraction, and ether and water then added, the ether took up the carotin and traces of α xanthophyll, while some of the α xanthophyll remained dissolved in the aqueous alcohol. Normal chloroplasts were found by KOHL to contain little α xanthophyll and little β xanthophyll while autumn leaves contained little α xanthophyll and much β xanthophyll.

Now, practically every word of the above description of the two xanthophylls is applicable to pigments which are well known today. One of these pigments, α xanthophyll, is now called xanthophyll while the data above for β xanthophyll exactly describes what are known today as the flavones. It is apparent then that no further discussion is needed regarding the two xanthophylls, α and β , described by KOHL.

THE XANTHOPHYLLS OF SCHUNCK

In a paper by SCHUNCK (6) it is reported that two yellow coloring matters were found accompanying chlorophyll in crude alcoholic extracts of healthy green leaves. The one was called chrysophyll (carotin) and the other xanthophyll (a pigment not readily adsorbed by charcoal). A third yellow pigment was sometimes found along with the xanthophyll. This pigment gave no absorption bands, but only an obscuration in the violet and ultra-violet region of the spectrum. In the latter case separation could be effected by ether. Evidence showed that other yellow coloring matters existed. Xanthophyll was believed by him to be the predominating yellow coloring matter accompanying chlorophyll in the healthy green leaf. Chrysophyll (carotin) and xanthophyll each gave characteristic absorption bands in the violet and the ultra-violet region, the former consisting of three bands and the latter of four, but in slightly different positions.

It is certain that SCHUNCK was dealing here with carotin, xanthophyll and the flavones as we know them. In addition, he seems to have been dealing with other yellow pigments, probably oxidation products of carotin and xanthophyll.

In another paper by SCHUNCK (7) three xanthophylls, L, B, and Y, are described. Alkalies appeared to have no effect upon the members of this group and saponification did not appear to alter them. They were never obtained in the crystalline form but each was found to possess three absorption bands. These pigments were distinguished by different spectroscopical reactions with acids. Hydrochloric acid had no immediate effect upon the spectral absorption bands of L xanthophyll, but produced an immediate and striking effect upon the spectra of B and Y xanthophylls, their solutions becoming paler in color. The solutions then became greenish blue and

finally colorless. L xanthophyll was more soluble in carbon disulphide than the B and Y forms.

Summarizing the properties of L xanthophyll as given by SCHUNCK may assist us in ascertaining the true nature of the pigment. Hydrochloric acid had no immediate effect upon L xanthophyll, but the bands faded after a time. Nitric acid caused the color of the pigment to fade at once. The L xanthophyll was more soluble in carbon disulphide than were the B and Y forms. The L form was more or less stable, its alcoholic solution showing but little change even after several weeks when kept away from the light. With hydrochloric acid no color reaction was produced in either alcoholic solutions of chrysophyll or those of L xanthophyll. Towards acids, hydrogen peroxide and nascent hydrogen, chrysophyll and L xanthophyll behaved very similarly although chrysophyll withstood the action of hydrochloric acid to a greater extent. The absorption bands of the two are very similar, a slight shift being the only difference.

All of the properties reported for L xanthophyll, in the above paragraph, are in perfect accord with those for carotin and there is no doubt that the L xanthophyll described was carotin. The only reason why SCHUNCK did not call the two pigments (L xanthophyll and chrysophyll) identical appears to be due to the fact that L xanthophyll was never obtained in the crystalline state. He remarks that "in none of the flowers experimented with was any chrysophyll obtained." Today we know that it is highly probable that carotin is present in all of the twenty flowers with which he worked, since the experience of the writer has shown that carotin often may be present in plant materials but may not always be obtainable in the crystalline form.

Our attention will now be directed to his B and Y xanthophylls. SCHUNCK found that B xanthophyll greatly exceeded Y in the tissues examined. The bands of the xanthophylls exhibited a gradual shifting toward the violet, those of chrysophyll being the least refrangible, the order being chrysophyll, L, B, and Y xanthophyll. On standing, the three xanthophyll extracts generally gave an indication of a fourth more refrangible band. Practically the only distinction SCHUNCK made in his B and Y xanthophylls was the slight difference in the position of the absorption bands. He always speaks of B and Y xanthophyll having certain properties and these properties in general agree very closely with those for xanthophyll. Also, he does not mention having found B and Y in the flowers of the same plant, all of which tends to show that the two are the same. The evidence then seems to show that SCHUNCK was working with carotin and xanthophyll, the L xanthophyll being carotin and the B and Y forms being xanthophyll. Oxidation of the pigments, too, must have played a part in his observations (8).

THE XANTHOPHYLLS OF TSWETT

TSWETT (9) apparently deals with several different xanthophylls. To obtain a clear idea of his work it will perhaps be best to start with the extraction of the pigments from the leaves according to his procedure (8, p. 321). Fresh leaves, preferably *Lamium*, were ground with fine emery in a mortar. Extraction was accomplished with petroleum ether which contained 10 per cent. alcohol. The green solution was then washed repeatedly in a separatory funnel with double its volume of water. After the turbid solution was filtered or centrifuged it was suitable for adsorption experiments. If the petroleum ether chlorophyll solution was shaken with precipitated calcium carbonate the pigments were precipitated, and with an excess of the precipitating agent only carotin remained in solution. If the carotin solution was shaken with 80 per cent. alcohol, the aqueous alcoholic phase remained colorless. The pigments were then completely removed from the precipitating agent by means of alcohol containing petroleum ether. After treating the bluish-green solution with 80 per cent. alcohol, the bluish tinged petroleum ether solution contained the chlorophyll while the alcoholic layer contained chiefly the xanthophyll.

If to the original petroleum ether solution of chlorophyll the adsorption agent was added gradually till the fluorescence disappeared, then the xanthophyll as well as the carotin remained in solution. The xanthophyll was freed of carotin, by treating the carotin-xanthophyll solution with an excess of the adsorption agent. The pigment xanthophyll was extracted from the adsorption medium by means of alcoholic petroleum ether. The xanthophyll mixture thus obtained showed absorption at 480–470 $m\mu$ and 452–440 $m\mu$. If shaken with 80 per cent. alcohol the pigment remained almost completely in the alcoholic phase.

From the above description there is no doubt that TSWETT was really dealing with carotin and xanthophyll, for the solubility in ethyl alcohol and the absorption in the violet region both indicate xanthophyll; and the manner in which the carotin solution was obtained shows us clearly that his pigments were carotin and xanthophyll as we know them today from WILLSTÄTTER's work. One point may well be taken up here; from the foregoing description of the method of preparing the pigments for use in the adsorption work it is very evident that TSWETT was never at any time dealing with pure pigments for not once were the substances crystallized, nor did he report any attempt made at crystallization. It may be assumed then that each of the solutions (carotin and xanthophyll) contained considerable traces of the other yellow pigment as well as large amounts of oxidation products of both of the pigments. This point must be clearly evident to any one who has isolated crystals of carotin or xanthophyll from their

respective carotin (petroleum ether) and xanthophyll (alcohol) fractions, and has noticed the large amount of noncrystallizable material remaining.

The chlorophyll mixtures used by TSWETT in his adsorption apparatus were generally obtained as described below: The leaves were ground with fine emery and a little calcium carbonate or magnesium oxide as neutralizing material, and were then extracted by means of alcoholic petroleum ether (1:10), after which the alcohol was carefully washed out of the petroleum ether by means of distilled water. The washing here was very thoroughly done, otherwise the alcohol and the water interfered with the adsorption bands of the chromatogram. Often the neutralized ground leaves were extracted with alcohol, acetone, ether or chloroform, which was distilled off in a vacuum and then carbon disulphide was used to again dissolve the pigments. The carbon disulphide solution of the pigments was used to obtain the chromatogram.

TSWETT recognized seven zones in the chromatogram and described them as follows, beginning at the top:

Zone I—Colorless.

II—Yellow, xanthophyll β , an alcoholic solution of the pigment absorbed in the regions 475–462 $m\mu$ and 445–430 $m\mu$. The pigment remained in the alcoholic phase of an aqueous alcoholic, petroleum ether system. Hydrochloric acid turned the alcoholic solution blue. All of the pigments of the various zones may be washed out quite readily. Ten per cent. alcoholic petroleum ether washed out xanthophyll from the adsorption medium.

III—Chlorophyll β .

IV—Chlorophyll a .

V—Yellow. Xanthophyll a^I and a^{II} . If benzene is poured into the tube containing the zones of color, zone V washes out slowly and forms a double ring.

Accordingly TSWETT assumed that two xanthophylls were present. The absorption spectrum of zone V was pushed a little further toward the ultra-violet than was the spectrum of xanthophyll a .

VI—Colorless.

VII—Orange yellow. Xanthophyll a . This pigment remained in the alcoholic phase of an aqueous alcoholic petroleum ether mixture. It bleached when hydrochloric acid was added and did not become blue. The position of the bands was 485–470 $m\mu$ and 455–440 $m\mu$ in an alcoholic or petroleum ether solution. When benzene was poured into the tube, zone VII was washed out at once.

Carotin passed through without being adsorbed. The carotin remained in the petroleum ether layer of an aqueous alcoholic petroleum ether mixture. The addition of concentrated hydrochloric acid did not turn its alcoholic solution blue. The absorption of the carbon disulphide solution was 525–510 $m\mu$, 490–472 $m\mu$ and weakly at 460–455 $m\mu$. In petroleum ether the bands were located at 492–475 $m\mu$ and 460–455 $m\mu$.

Only the portion of TSWETT's work which refers to the yellow pigments has been quoted in full for it is with the yellow pigments alone that this paper is concerned. We are concerned not with the carotin fraction but with the various xanthophylls which he has described. It will be observed that impure solutions of pigments were used in all of the work. Large amounts of oxidized products of both carotin and xanthophyll undoubtedly were present in the pigment mixtures used.

THE WORK OF PALMER AND ECKLES AND TSWETT'S CHROMATOGRAM

Perhaps PALMER and ECKLES (4) have done more with TSWETT's absorption column and his methods than any other worker or group of workers. The yellow pigments were separated from the butter fat by saponifying for $\frac{1}{2}$ hour with a 20 per cent. solution of alcoholic potash, at the temperature of the boiling solution. Three volumes of distilled water were added and the pigment was shaken out with ether, which was then washed and dried. The plant pigments of alfalfa were extracted by adding carbon disulphide to the ground material and allowing it to stand several days.

In all of the experiments on butter fat and alfalfa it is noteworthy that a carotin-like substance passed through the calcium carbonate in the chromatogram. A xanthophyll-like substance was adsorbed somewhat and then there was a yellowish substance which could not be removed at all by carbon disulphide, but could be removed by alcoholic petroleum ether from the chromatogram. In the case of butter fat (3, p. 352) the portion which passed through constituted about 98 per cent. of the total pigment. The adsorbed portion consisted of approximately 2 per cent., while about 8 per cent. of the portion which passed through was alcohol soluble. The authors do not seem to make any distinction as to whether the pigments are fractionated by dissolving them in a petroleum ether solution and then fractionating by means of alcohol, or by dissolving them in alcohol and then fractionating by adding successive portions of petroleum ether. The manner of fractionating would make a great difference in the quantitative results, for by extracting the alcoholic solution, the xanthophyll fraction would appear to be less than it really is, and, besides, part of the xanthophyll would appear to be carotin. (The proper method of separating carotin from xanthophyll is to fractionate a petroleum ether solution of the mixed pigments repeatedly with aqueous methyl alcohol.)

PALMER and ECKLES in their feeding experiments to determine the pigments present in butter fat, studied the pigment content of the feeds used. In the unsaponifiable pigments (3, p. 361) of cottonseed meal they found equal parts of carotin and xanthophyll, the xanthophyll being made up of at least five different constituents according to their adsorption properties. The chief xanthophyll is not adsorbed to any extent by calcium carbonate from its carbon disulphide solution, which also shows in the spectroscope a shifting of the bands toward the blue, from the normal xanthophyll bands. The remainder of the xanthophylls were held so firmly by the calcium carbonate that they could not be readily removed by a stream of carbon disulphide. One per cent. alcoholic petroleum ether would easily wash all of them out.

The yellow pigments of yellow corn (3, p. 362) were not adsorbed from either petroleum ether or carbon disulphide by calcium carbonate. Petroleum ether readily extracted the pigment from its concentrated 80 per cent. alcoholic solution but it could be completely reextracted from its petroleum ether solution by fresh 80 per cent. alcohol. In this respect it differed from any xanthophyll-like pigment yet investigated. The minor constituents of the corn pigments, as to solubility, spectroscopic, and adsorption properties resemble carotin. A carbon disulphide solution of yellow pigments from carrots was passed through the chromatogram. Only a small portion of the pigment was adsorbed and this was not differentiated into zones. It was readily washed out with alcoholic petroleum ether. The xanthophyll here was only 3-4 per cent. of the entire yellow pigments. Zone I was entirely different from all of the others; its alcoholic solution turned bluish-green when concentrated hydrochloric acid was added. This xanthophyll the authors believed to be identical with xanthophyll β of TSWETT and xanthophyll β of SCHUNCK.

Since the authors usually extracted the yellow pigments from alcohol by means of petroleum ether, not much reliance can be placed upon their quantitative data regarding the amount of carotin or xanthophyll present in the various substances.

All of the xanthophylls reported in this paper have already been correlated except those described by TSWETT, GILL, and PALMER and ECKLES and these will now be considered.

EXPERIMENTS WITH PURE XANTHOPHYLL AND PURE CAROTIN

Investigation showed that by using practically pure solutions (5) of xanthophyll only a very little, or none, of the pigment was absorbed when a carbon disulphide solution of xanthophyll was passed through a tube filled with calcium carbonate. If the carbon disulphide solution after being passed through a calcium carbonate column was allowed to stand for a day or more,

and then again put through the tube, more of the pigment was adsorbed. Usually the longer the carbon disulphide solution of xanthophyll stood, exposed to air and sunlight, the greater would be the amount of the adsorbed yellow pigment. This adsorbed yellow pigment, when washed out by means of 95 per cent. ethyl alcohol, and taken up in petroleum ether, may be wholly extracted from the petroleum ether by means of 92 per cent. methyl alcohol; hence it had the properties of xanthophyll.

Carotin behaved in approximately the same way that xanthophyll did. Pure solutions of both in carbon disulphide left only traces of yellow pigment in the adsorption column, while solutions which were partially oxidized left a band of yellow in the calcium carbonate tubes. Xanthophyll always left much more of the adsorbed pigment behind.

An experiment may serve to show the relative properties of the two pigments. After adding pure solvent (CS_2) to an adsorption tube, 5 cc. of pure carotin solution was added. In about 4 minutes, and by adding 10 cc. of pure carbon disulphide, all of the carotin had passed through the column, save a mere trace. An equal amount of xanthophyll solution of approximately the same concentration as the carotin solution required more than 15 minutes and at least 50 cc. of carbon disulphide before the carbon disulphide passing through the adsorption tube became colorless. A yellow zone usually remained. This indicated in a way the relative speed with which the two pigments passed through a calcium carbonate column. It is certain that carotin passed through the column much more rapidly than xanthophyll did, but in a mixture of the two pigments it would be very difficult to say just when carotin has ceased coming through and when xanthophyll had begun. Or perhaps the period of the last traces of carotin and the first traces of xanthophyll might easily overlap each other and thus there would be no sharp separation of the two pigments by the adsorption method. The method cannot be recommended to distinguish carotin and xanthophyll. However, it might be of some value after considerable experience with it. On the other hand, the method of separating the two pigments by means of petroleum ether and methyl alcohol is absolutely reliable, easy to perform, even by beginners, and in every way is far more satisfactory than the adsorption method. There is little chance of making errors by this method.

EXPLANATION OF THE INCONSISTENCIES IN RECENT LITERATURE

There remains for us to make clear a few of the cases which have been cited above. In considering the regions or zones described by TSWETT, it is necessary to use caution for it is to be remembered that chlorophyll in any one of its many forms may appear in the different zones and cause color changes which are very misleading.

Zone II (xanthophyll β) of TSWETT is undoubtedly composed of oxidized xanthophyll, since pure xanthophyll would wash out by further washing with carbon disulphide. Zone V (xanthophyll α^I and α^{II}) and VII (xanthophyll α) are apparently made up of pure or partially oxidized xanthophyll, but an explanation of why it separates into zones here, is another matter. Since stages in the oxidation of xanthophyll have not been studied, nor the oxidation of xanthophyll ever controlled, it is not possible to say definitely that these zones are stages in the oxidation of xanthophyll; but the fact that the xanthophyll separates into zones and that oxidation of xanthophyll causes a band to be found in the adsorption column, all lead us to suspect that the phenomenon of TSWETT is probably the result of different stages of oxidation of xanthophyll.

The yellowish substance, which could not be washed from the chromatogram by carbon disulphide and which PALMER and ECKLES found in butter fat, is undoubtedly an oxidized carotinoid. The chief pigment of cottonseed meal was found to be xanthophyll (by PALMER and ECKLES) which was not adsorbed by calcium carbonate. This non-adsorption is not surprising, for pure xanthophyll is not adsorbed. The yellow corn pigment was not adsorbed by calcium carbonate, and petroleum ether extracted the pigment from 80 per cent. alcohol; but the pigment in turn could be completely re-extracted from the petroleum ether by means of 80 per cent. alcohol. According to the authors it differed from all other xanthophylls in this respect. Since all of the above characteristics are those of pure xanthophyll, nothing is strange about the behavior of the pigment. In the case of the carotinoids from carrots only a small portion of the pigment is adsorbed, which was exactly what would have been expected with carotin.

The statement by GILL is perhaps the most difficult to harmonize with the properties of the yellow pigments. He states that in certain cases (yellow corn, mustard, and orange peel) none of the pigments is extracted by 80 per cent. alcohol from petroleum ether. This would indicate carotin in all 3 plant materials. Further he says that large amounts are adsorbed by calcium carbonate; this would indicate xanthophyll. Since oxidized xanthophyll behaves in regard to alcohol and petroleum ether in the same manner as pure xanthophyll, the adsorbed portion could not have been xanthophyll nor its oxidation product. The only other possibility is that it is oxidized carotin, since pure carotin would not be adsorbed, and this is not possible in the case of the pigment of yellow corn, which was one of the cases in question; the pigment present in yellow corn is known to be xanthophyll with only a trace of carotin. Since PALMER and ECKLES give exactly the opposite properties regarding the adsorption of the chief pigment of yellow corn and since the properties of this pigment as they describe it agrees with

those of xanthophyll it is assumed that the observations of PALMER and ECKLES are correct.

The pigment extracted from orange peel has been investigated by the writer and it was found that none of the pigment was adsorbed from a carbon disulphide solution by calcium carbonate, while none or only a very little was extracted from petroleum ether by 80–92 per cent. methyl alcohol. This would indicate that the greater portion or nearly all of the pigment in orange peel is carotin. Here again it is shown that adsorption of the pigment is questioned, for the observations reported here, and those of GILL who used TSWETT's methods are contradictory. However, observations made in this investigation and those of PALMER and ECKLES, regarding the distribution of the pigment between petroleum ether and methyl alcohol agree. These facts show again the unreliability of the method described by TSWETT for distinguishing carotin and xanthophyll, and also that the methods of WILLSTÄTTER may be relied upon.

Summary

1. A brief summary is given of the work on the yellow pigments.
2. Certain apparent inconsistencies in the literature are pointed out and published statements regarding the carotinoids are shown to harmonize with known data for xanthophyll and carotin.
3. The xanthophylls (α and β) of KOHL are shown to be xanthophyll and flavone respectively.
4. The chrysophyll, xanthophyll, and the third yellow pigment of SCHUNCK are shown to be carotin, xanthophyll, and flavone respectively. SCHUNCK's L xanthophyll apparently is carotin, while his B and Y xanthophylls are xanthophyll.
5. TSWETT's xanthophyll β apparently consists of oxidized xanthophyll. His xanthophylls α , α^I and α^{II} , from his descriptions, have properties very similar to those for pure xanthophyll, and most probably result from slight oxidation of the pure pigment.
6. Pure carotin has been shown to be unadsorbed by calcium carbonate. Pure xanthophyll is only relatively adsorbed, that is, it passes through an adsorption column more slowly than does carotin.

While TSWETT's methods have been shown to be unreliable in identifying and distinguishing carotin and xanthophyll, the methods of WILLSTÄTTER have been shown to be more dependable for this purpose.

LITERATURE CITED

1. GILL, AUGUSTUS H. Occurrence of carotin in oils and vegetables. *Jour. Ind. Eng. Chem.* **10**: 612-614. 1918.
2. KOHL, F. G. Untersuchungen über das Carotin. 206 pp. Leipzig. 1902. (See pages 140-142.)
3. PALMER, LEROY S., and ECKLES, C. H. Carotin, the principal natural yellow pigment of milk fat. Part II. *Univ. Missouri Res. Bull.* no. 10. 339-387. 1914.
4. ————. Carotin, the principal natural yellow pigment of milk fat. *Res. Bulls.* nos. 9, 10, 11 and 12. *Univ. of Missouri.* 1914.
5. SCHERTZ, F. M. Some physical and chemical properties of xanthophyll and the preparation of the pure pigment. *Jour. Agr. Res.* **30**: 575-585. 1925.
6. SCHUNCK, C. A. The yellow coloring matters accompanying chlorophyll, and their spectroscopic relations. *Proc. Roy. Soc. London B* **65**: 177-186. 1899.
7. ————. The xanthophyll group of yellow coloring matters. *Proc. Roy. Soc. London B* **72**: 165-176. 1903.
8. TSWETT, M. Physikalisch-chemische Studien über das Chlorophyll. Die Absorptionen. *Ber. deutsch. bot. Ges.* **24**: 316-323. 1906.
9. ————. Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. *Ber. deutsch. bot. Ges.* **24**: 384-393. 1906.
10. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR. Untersuchungen über Chlorophyll. Berlin. 1913.

GROWTH STUDIES ON FRUITS. RESPIRATION OF TOMATO FRUITS¹

FELIX G. GUSTAFSON

(WITH THREE FIGURES)

The measurements of respiration here reported have been made as a part of a general study of the growth of tomato fruits. The fruits used have partly come from plants grown in the open during the regular growing season and partly from plants grown in the greenhouse during the winter. The plants grown in the greenhouse have been supplied with additional light during the period of shortest days. The fruits from these plants have been normal in all respects so that comparisons can be made between fruits grown out of doors and indoors.

The experiments have been conducted and the amount of carbon dioxide determined as reported by HOVER and GUSTAFSON in another paper (6), except that the length of time of each experiment has been much shorter. In all of the experiments cited only one fruit was used in each container, so that the figures given are not averages for several fruits, but rather values for individual fruits. In all of these experiments the fruits were picked from the vines about a half hour to an hour before the experiment was started.

The first experiments were conducted in the fall of 1927 on Livingston Globe and John Baer tomatoes grown in the field. The fruits used were of known age. Once a week during the summer flowers that had just opened were tagged. This supplied me with fruits differing in age by one week, ranging from one week old to six, seven or eight weeks, or mature fruits.

In these first experiments fruits of different ages were selected, and no attention was paid to state of development or ripeness. Fig. 1 illustrates the results obtained with Livingston Globe. The John Baer fruits gave similar results, which were not quite as complete and for that reason are not included in this figure. In experiments A and B the youngest fruits were 8 and 9 days old, respectively, and the other fruits 1, 2, 3, 4, and 5 weeks older. It is to be noted that as the fruits become older the respiration decreases rapidly at first and then more slowly. Experiment C, which was conducted 4 days later than B, shows that the respiration of the youngest fruit was lower than in the preceding experiments but it agreed with what one would be led to expect from the curves A and B. Experiment D, which was conducted a week later than C, or when the youngest fruits were 3 weeks old had a rate consistently somewhat lower than expected, but this may have been due to a lower temperature.

¹ Paper from the Department of Botany of the University of Michigan, no. 301.

It will be noted that at no time was there an actual increase in respiration, but always a decrease. Several years ago HOVER and the writer noted that as leaves of sorghum, corn, oats and wheat matured, *i.e.*, aged, there came a period of minimum respiration after which there was an increase. I had expected to find a similar condition for fruits also but, as the curves show, it was not realized.

For a while it was thought that the difference in respiration rate of fruits of different ages might be due to size; *i.e.*, small fruits having a relatively larger surface than large fruits, might possibly respire more per gram through which the absorption of oxygen and excretion of CO_2 takes place. In the fall and winter of 1927 experiments were therefore conducted to determine whether the size of the fruit exerted any influence on the rate of respiration, but nothing definitely was ascertained though in some experiments it seemed that large fruits respired less per gram of material than smaller fruits of the same age. Again, during the summer of 1928, this problem was taken up and this time the area of the surface was measured.²

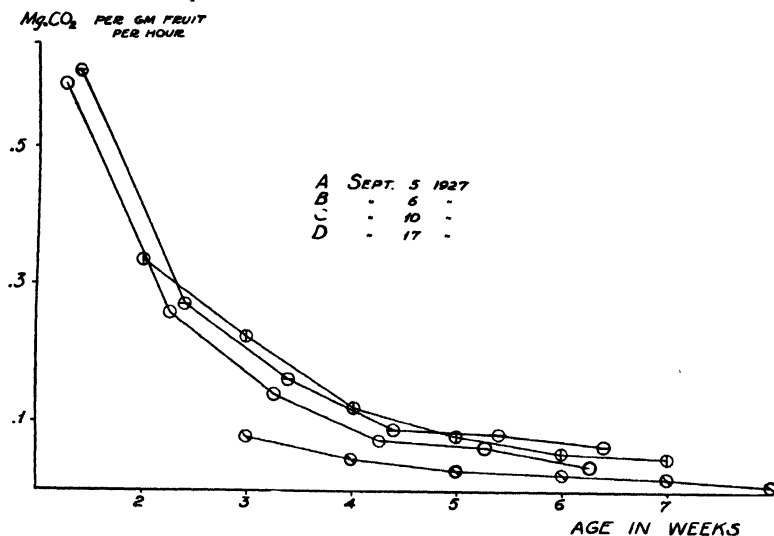


FIG. 1. Respiration of Livingston Globe tomato fruits. In this figure as well as in those to follow the green weight is used.

² The area of the surface was obtained by using SIMPSON'S 1/3 rule, where:

$$\text{Surface} = 4 C \times \frac{1}{3} S$$

S is one fourth of any circumference of the fruit passing through the point of stem insertion. C is the circumference of the fruit passing through points situated S distance from the center of the fruiting stem. The value thus obtained is 4.84 per cent. too large and has been corrected by that much reduction. I am indebted for this formula to Professor THEODORE R. RUNNING of the Mathematics Department of the Engineering School at the University of Michigan.

Fruits of the same age but having a different $\frac{\text{area}}{\text{weight}}$ ratio were used. When the weight of CO_2 produced was plotted against this ratio there was no uniformity and so far I have no evidence which would show that the increased surface exposed increases respiration. During the conduction of these experiments it was, however, discovered that although the fruits may be of the same chronological age, yet they may be of very different age physiologically. The recognition of this fact complicates the matter of relation between $\frac{\text{area}}{\text{weight}}$ ratio and rate of respiration, because it is next to impossible to get a number of fruits of exactly the same physiological age yet having different $\frac{\text{area}}{\text{weight}}$ ratios.

Finding that it would be very difficult to determine the effect of varying the $\frac{\text{area}}{\text{weight}}$ ratio on the rate of respiration this factor was eliminated with considerable success by choosing fruits of the same $\frac{\text{area}}{\text{weight}}$ ratio but of different physiological ages.

In the next experiments the chronological age was ignored, and fruits of approximately the same size but of different physiological ages were chosen. In this group of experiments only maturing fruits were used and after some study the physiological age of a fruit could be determined quite accurately from its color.

In fig. 2 are plotted the results of such experiments. The terms used on the abscissa are the color changes through which these fruits pass when ripening. It is realized that they are not very accurate but they are the best available. When 6 or 7 fruits differing only slightly in their physiological age, as shown by difference in color, are used in the same experiment, it becomes obvious that during the ripening period there is an increase in respiration which in turn is followed by a decrease as the fruits ripen still more. The increase is not very great in actual value but in terms of percentage it is quite considerable. Thus in experiment C the increase is 95 per cent., in D 75 per cent., while A and B show about 10 per cent. increase. These experiments were conducted in September, 1928. In the October number of the Proceedings of the Royal Society of London there appeared a paper by F. F. BLACKMAN (2) in which he describes experiments on stored apples, where the same phenomenon was noticed.

It is one thing to determine the physiological age or state of activity in a maturing fruit in which there are definite color changes and a very different thing to determine it in a green fruit. Age and size mean nothing. The way I have attempted to get at this is by measuring the diameters of the fruit at short intervals. In this way I have attempted to correlate rate of

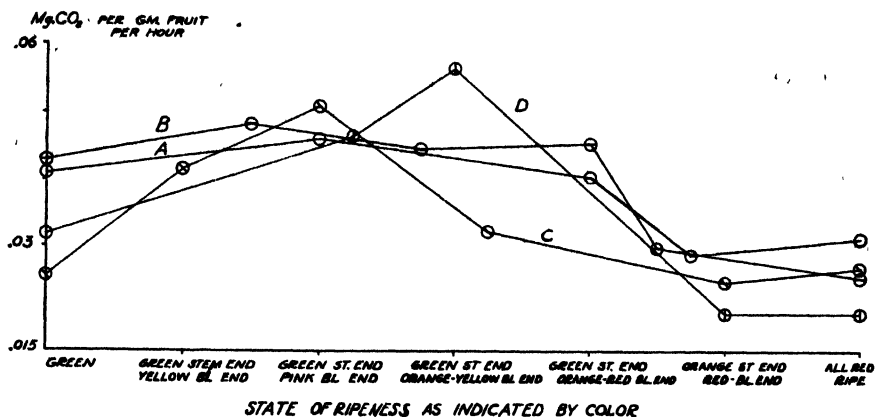


FIG. 2. Respiration of ripening John Baer tomato fruits, showing that as the fruits mature there is an increase in the rate of respiration, which is later followed by a decrease. The increase in respiration in experiments A and B was for each one about 10 per cent., for C 95 per cent. and for D 75 per cent.

respiration with the position a fruit occupied on the growth curve, but no two fruits have exactly the same growth curve and the placement of a fruit might be somewhat arbitrary, therefore in the calculations as shown in fig. 3 I have plotted amount of CO₂ against percentage increase in diameter per day of the fruits. Two diameters at right angles to the axis passing through the stem of the fruit were measured usually twice a week. From these data growth curves were plotted for each fruit and the fruits to be used in an experiment were selected with reference to their position on the growth curve.

In his paper on growth of the tomato MACDOUGAL (7) points out that the rate of increase in diameter is not a correct measure of actual growth, but rather that growth varies as the cube of the radius. Some of my data have been calculated in terms of percentage increase in the cube of the radius, but as the shape of the curve is the same, time has been saved by using the percentage increase in diameter in all experiments given in fig. 3.

When fruits for study were selected in this way curves like those represented by fig. 3 were obtained. These curves show a high rate of respiration when increase in diameter is large and a minimum when there is no longer an increase in size but the fruits are still green. This minimum was followed by an increase which reached its maximum at about the time when the fruits were of an orange to red color with the stem end still green. As in previous experiments with ripening fruit the actual increase is not very large but in terms of percentage it ranges from practically zero to 123 per cent.

These last experiments then show that when the tomato fruits are actively growing the respiration is high (in some instances as high as 0.75 mg. of CO_2 per gm. fresh fruit per hour). As growth diminishes there is a decrease in respiration which reaches a minimum at about the time the fruits stop increasing in diameter, to be followed by an increase, as the maturing process progresses, to a maximum at the time the fruits are orange to red in color. A second decrease continues until the fruits are ripe, when there is very little respiration.

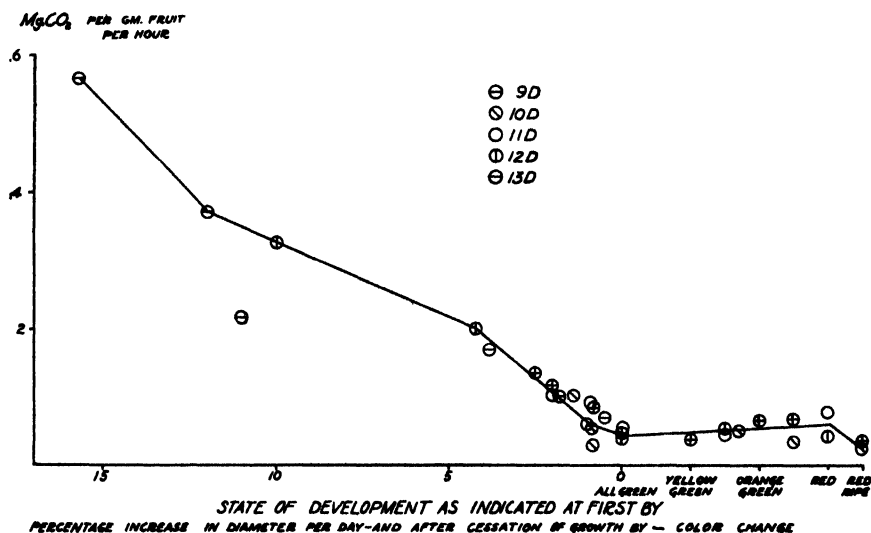


Fig. 3. Respiration of John Baer tomato fruits at different physiological ages.

These experiments showed that tomato fruits like leaves and apples have a period of minimum respiration which is followed by an increase as the fruits mature. Like apples, but as far as we know unlike leaves, this increase is further followed by a decrease.

It is to be expected that the young actively growing fruits will have a high respiratory rate, because respiration is so very closely connected with the life activity of an organism. It is also only to be expected that as the fruits become older, and growth slows down, respiration will diminish. It is, however, very hard to understand why there should be an increase in respiration after growth, *i.e.*, enlargement, has stopped. BLACKMAN explains this in the apple by assuming that as the fruits ripen there is a "lowering of the grade of organization-resistance" or more specifically a lowering of "hydrolysis resistance," which means that hydrolysis is facilitated during the ripening period. This increases the concentration of substances

utilized in respiration, and respiration increases. The subsequent decrease, he thinks, is due to starvation. Possibly there is starvation in apples that have been stored for several months, but certainly not in ripening tomatoes. Both ALBAHARY (1) and SANDO (8) have shown that there is an increase in sugars during the development of the tomato fruit.

According to Sando the percentage of total sugar in a ripe tomato is nearly twice that of a two-weeks-old fruit. He found that during the last two weeks there was an increase from 42.23 to 48.32 per cent. as calculated on the basis of dry weight or from 2.375 to 2.667 per cent. on the basis of green weight, and the red fruits have a sugar content which is two per cent. higher than that of the fruits turning color; but according to my findings the red ripe fruits with the higher sugar content respire less. In tomato therefore the final decrease in respiration cannot be due to starvation.

The decrease in respiration in the mature fruit might rather be attributed to complete cessation of all activity. The mature fruit is essentially water and carbohydrate. According to my own data (3) Livingston Globe 9 weeks old (nearly mature) contained only 4.6 per cent. dry material, John Baer 8 weeks old (mature) contained 5 per cent. dry material. SANDO found 8 weeks old (red) Livingston Globe to contain 5.5 per cent. dry material. Of this dry material carbohydrate made up 62.45 per cent., crude ash 9.14 per cent. and protein 13.13 per cent. Only 0.725 per cent. of the fresh fruit was protein and a great deal of this is in the seeds. In a system like this it is not very likely that life processes are very active. The whole system is slowly running down.

To return to the increase in respiration following the minimum point reached at about the time the green fruits stop enlarging, if augmentation in hydrolysability and the consequent augmentation in material utilizable in respiration is a factor in this increase of respiration it would seem that respiration should increase continuously from the beginning of the fruit development till the end, because, according to SANDO, the percentage of sugar content is constantly becoming greater, at least after the fruits are two weeks old. The percentage of protein is constantly diminishing, so respiration cannot be associated with that either.

In a recent paper (4) it has been shown that there is an increase in the hydrogen ion concentration as the fruits enlarge until a point of maximum concentration is reached, after which there is a decrease. In the experiments referred to, 50 fruits were used for each determination and these were average fruits of a given age, so that they varied in physiological age. At the time the maximum hydrogen ion concentration was reached in the fruits a few were beginning to turn but most were still green. This fact corresponds very well with the condition of the fruit at the point of minimum respiration. As it is a well established fact that respiration is

very greatly influenced by the hydrogen ion concentration (5) it seems to me that the explanation for the increase in respiration of the maturing tomatoes might be the decrease in the hydrogen ion concentration of the cell sap. This is merely a suggestion, but when the hydrogen ion concentration curve is compared with the respiration curve, it seems that this must be the correct answer. The origin of the acid substances need not concern us. They may be the result of the respiration itself, in which case we would have a self-regulatory reaction.

A word may also be said about the use of the physiological age, as contrasted with chronological age. As has been pointed out elsewhere, and as every one who has studied tomatoes knows, some fruits do not begin to develop at once after the flowers wither, but only after a week or two of inactivity. Physiologically these fruits are younger than the ones that started to grow at once. Frequently there are fruits in a cluster which, for one reason or another, get a slow start and grow very slowly until the other fruits are either mature or have been picked. Then they begin to grow very rapidly and consequently are much younger physiologically than chronologically. In a population of tomato fruits as they exist in a field one has to deal with such cases and for that reason age as measured in days and weeks is not a fair criterion of the behavior of a fruit. As far as I know this distinction has not been made in plant physiology, but it is one with which we are all familiar, at least in the human family. Not all people act their age.

Summary

1. It has been shown that there is a decrease in the production of CO_2 by John Baer tomato fruits during growth, until a point of minimum production is reached at about the time increase in size stops; that this is followed by an increase in CO_2 production which reaches its maximum when the fruits are orange to red in color and that thereafter there is a final decrease in the rate of respiration.

2. It has been suggested that the increase is due to a lowering of the hydrogen ion concentration of the cell sap.

3. The final decrease in CO_2 production is thought to be due to a slowing down of all life activities as the tomato approaches a condition of a mixture of carbohydrate and water.

4. It has been pointed out that sometimes there is a difference between the chronological and physiological age in tomatoes, and that the latter should be used when various activities are studied.

UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN.

LITERATURE CITED

1. ALBAHARY, F. M. Étude chimique de la maturation du *Lycopersicum esculentum*. Compt. Rend. Acad. Sci. Paris. **147**: 146-147. 1908.
2. BLACKMAN, F. F. Analytic studies in plant respiration. I. The respiration of a population of senescent ripening apples. Proc. Roy. Soc. London, B. **103**: 412-445. 1928.
3. GUSTAFSON, F. G. Growth studies on fruits. Plant Physiol. **1**: 265-272. 1926.
4. ————. Growth studies on fruits. Chemical analyses of tomato fruits. Papers Mich. Acad. Sci., Arts and Letters. **8**: 121-127. 1927.
5. ————. Comparative studies on respiration. XI. The effect of hydrogen ion concentration on the respiration of *Penicillium chrysogenum*. Jour. Gen. Physiol. **2**: 617-626. 1920.
6. HOVER, J. M., and GUSTAFSON, F. G. Rate of respiration as related to age. Jour. Gen. Physiol. **10**: 33-39. 1926.
7. MACDOUGAL, D. T. The physical factors in the growth of the tomato. Bull. Torrey Bot. Club. **47**: 261-269. 1920.
8. SANDO, C. E. The process of ripening in the tomato, considered especially from the commercial standpoint. U. S. Dept. Agr. Bull. 859. 1920.

SOME OBSERVATIONS ON THE MICROCHEMICAL DEMONSTRATION OF PHLORIDZIN¹

E. M. HARVEY

(WITH THREE FIGURES)

MOLISCH (2), in his "Mikrochemie der Pflanze" (p. 162) states: ". . . Although the number of known glucosides in plants is exceedingly large, yet one is able to demonstrate but a few microchemically. In such tests one seeks either to demonstrate the specific glucoside directly, or, after hydrolysis, to demonstrate the non-sugar constituent."

The writer believes that, in the case of phloridzin, a direct demonstration is the more feasible, seeing that phloridzin and phloretin, its non-sugar derivative, have precisely the same color reactions. Arbutin, on the other hand, may give different color reactions than its derivative hydroquinone.

Several reagents for demonstrating phloridzin have been suggested. Among the best are concentrated sulphuric acid, nitric acid, and ferric chloride. The last named is an excellent reagent and, with a little experience, offers still the best aid to the investigator. The writer attempted to find some other reagent which would be more specific for phloridzin, but none distinctly superior to ferric chloride was found among the several hundred substances or combinations tried. Some, however, seemed worthy of consideration, such as uranium acetate (or chloride), mercuric nitrate, aluminium nitrate, antimony pentachloride, and sodium tungstate. These were tested not only upon phloridzin, but also upon nineteen other glucosides or glucosidal derivatives and tannins. The color reactions resulting were frequently rather distinct and characteristic. For example, with *ferric chloride*, phloridzin gives a clear orange to vinaceous red; tannic acid gives a deep blue or blue-black; arbutin, a blue also; salicin is colorless; and quercitrin produces a deep green. With *uranium acetate*, phloridzin is orange, while tannic acid and arbutin give with this reagent a vinaceous red. With *aluminium nitrate*, phloridzin gives a lavender-pink to vinaceous red; tannic acid, a clear green; and arbutin is colorless. The employment of two or more reagents, with strict attention to the color reactions should aid greatly in the identification of certain glucosides. One should also take into consideration the dominance of one reaction over another. To illustrate, it was found by testing certain solutions with ferric chloride that tannic acid dominated phloridzin, and the latter the arbutin reaction. If one part phloridzin is present to ten parts arbutin, the blue coloration of arbutin will not be evident. One part phloridzin to twenty or thirty parts

¹ Published with the approval of the Director of the Experiment Station.

arbutin will still interfere somewhat with the latter reaction. On the other hand, one part of tannic acid to three parts phloridzin entirely obscures the phloridzin reaction; but with one part tannin to six or seven parts phloridzin, a green coloration results.

The above statements indicate some of the factors which must be considered in making microchemical tests for glucosides.

The writer has made many more or less sporadic microchemical tests for phloridzin in various species of Rosaceae. Except for the apple, phloridzin could not be positively identified in any of the species examined. Nevertheless its presence was strongly indicated in our choke cherry, *Prunus demissa*. Phloridzin has been reported from the sweet cherry and prune and other Rosaceae besides the apple, but such observations seem at present uncertain. HERMANN (1) states: "In the pear, prune and cherry

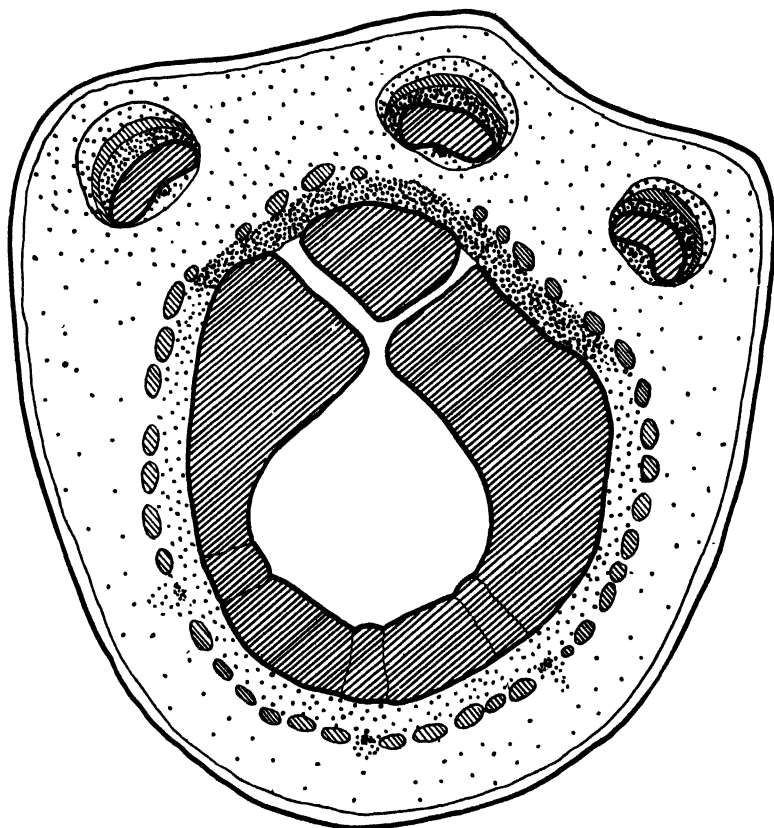


FIG. 1. Cross-section of apple shoot on March 16. Cut just below a leaf scar. The dots show the presence and relative amounts of phloridzin. Cross hatching indicates xylem and bast.

the phloridzin reaction does not occur, on account of the interference by tannic acid." But TUNMANN (see 1) comments on this interpretation: "Perhaps there is no phloridzin present."

In the apple the demonstration of phloridzin is a simple matter. Either ferric chloride or uranium acetate will serve as a reagent. A stock solution of ferric chloride (50 per cent.) will keep indefinitely. This is diluted to five, two, or even one per cent. for immediate use. These solutions may be used for direct examination of specific tissues by flooding cross or longitudinal sections on a slide, using a binocular microscope with strong illumination from above. Or, since the reaction is a "diffused" one, that

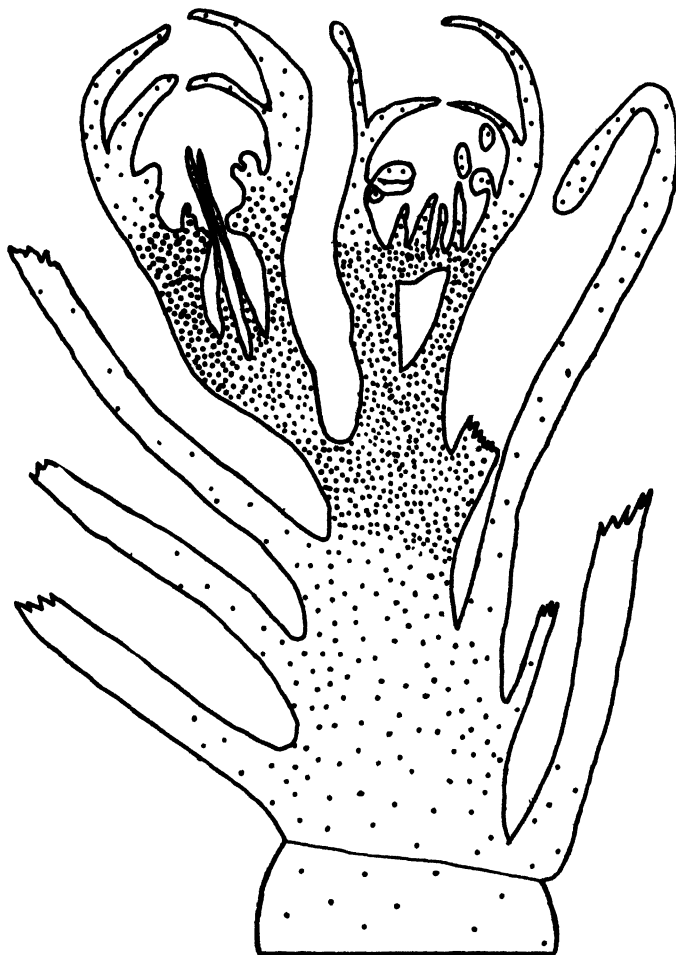


FIG. 2. Section through a bursting terminal fruit bud of the apple. The dots show the presence and relative amounts of phloridzin.

is to say one in which a vinaceous red cloud soon appears out from the margins of the sections, the fact may be taken advantage of in comparing different tissues. Representative pieces of outer or inner cortex or phloem, for instance, can be dissected out and tested separately, observing, on crushing in the reagent, the relative density and breadth of the diffusion clouds of phloridzin. As to the localization of phloridzin in apple tissue, HERMANN (1) reports the following: "Phloridzin is localized in the outer cortex, a little in the phloem region, and none in the cambium, bast or wood." Fig. 1 shows that this statement agrees fairly well with the findings of the writer. However, the following modification is suggested. Phlo-

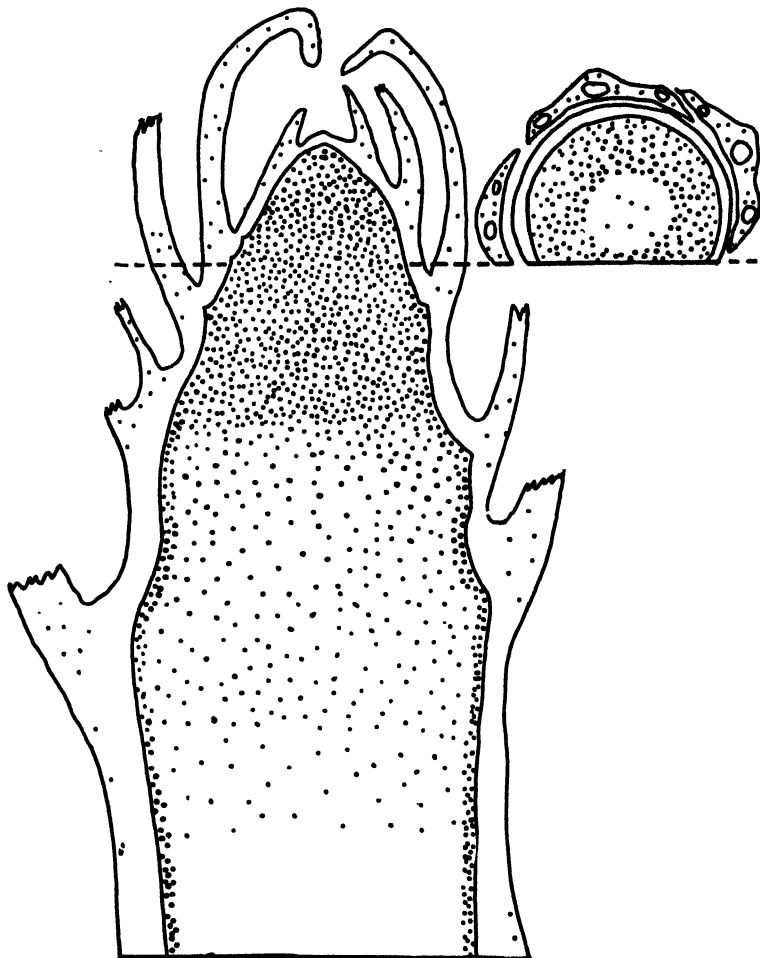


FIG. 3. Partial section through a bursting terminal vegetative bud showing the growing point. The dots indicate the presence and relative amounts of phloridzin.

ridzin is most abundant in the phloem, particularly the phloem of leaf traces, abundant in the outer cortex, or inner periderm, but rather scarce in the inner cortex, except at the nodes around the leaf traces, and if it does not occur in the cambium, it occurs very near it. Tannins cause the color reaction to be greenish in the outer cortex. In the bark of spurs and older branches, there is usually sufficient accumulation of tannins in the outer phloem rings to make the reaction unsatisfactory. Older roots, while containing large quantities of phloridzin, seldom give clear reactions on account of tannin.

At the beginning of growth activity in the spring, when buds are swelling or bursting, the bases of axillary and terminal buds are gorged with phloridzin. Then the inner bud scales, the peduncles, and receptacles of the embryonic flowers are also gorged with it (see fig. 2). A quantitative determination of phloridzin in styler tissue showed, on a dry weight basis, 15.20 per cent. At the same time the new growth of the spur contained but 8.42 per cent. phloridzin and the spur bark of the previous year, 5.98 per cent. Large quantities were observed microchemically in the growing points of bursting buds (see fig. 3).

The conclusion seems justified that phloridzin is localized and abundant in, or in close proximity to, the most active tissue.

AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

LITERATURE CITED

1. HERMANN, O. Nachweis einiger organischer Verbindungen in den vegetabilischen Geweben. Dissertation, Leipzig, 1876. *Cited by* TUNMANN, O. Pflanzenmikrochemie. Berlin, 1913.
2. MOLISCH, HANS. Mikrochemie der Pflanze, Jena, 1913.

THE EFFECT OF pH VALUE AND HYDROGEN PEROXIDE CONCENTRATION ON FRUIT OXIDASE ACTIVITY

W. V. CRUESS AND W. Y. FONG

In connection with our studies on the darkening of fruit tissue, investigations have been made on the effect of hydrogen ion concentration and hydrogen peroxide concentration on the behavior of the oxidase system of the apricot. Some of the observations that appear to be of general interest are presented in this paper.

Effect of pH value on the action of the oxidase on various indicators

Several investigators have proved that hydrogen ion concentration markedly affects the activity of various oxidases.¹ Among these may be mentioned UCKO and BANSI (7) who found that the pH optimum for the peroxidase of horse radish varied with the nature of the oxidase indicator, being pH 7 for pyrogallol and 5.2 for guaiacol; CLARK and ZOLLER (2) who selected a number of oxidation and reduction indicators for oxidizing enzymes; RAPER (6) who found that the maximum effect of tyrosinase or tyrosin occurs at pH 6-8 and that there is no action below pH 5 or above 10; and OVERHOLSER and CRUESS (5) who found that the activity of apple oxidase on benzidine was affected markedly by the pH value of the medium. OVERHOLSER (4) finds that pear catalase is most active in the neighborhood of pH 7.0.

Our measurements on the effect of the pH value of the medium on apricot oxidase were made both upon the natural apricot juice and on the enzyme purified by precipitation with alcohol and reprecipitation by acetone from water solution. Incidentally, acetone was found to be an excellent precipitant for fruit oxidase as the precipitate was granular and easily washed and filtered.

Portions of apricot juice were brought to various pH values ranging from pH 2.0 to 11.1 and a number of different oxidase indicators were added, together with a small measured amount of 0.3 per cent. H_2O_2 solution. The peroxidase was found in most cases to be capable of oxidizing α -naphthol at pH 2.8 but not at 2.6; ortho-amino-phenol, para-amino-phenol, hydroquinon, pyrogallol, para-phenylene-diamin hydrochloride and benzidine at pH 2.6 but not at 2.4; para cresol at pH 2.9 but not at 2.6; guaiacol at pH 3.0 but not at pH 2.8; tincture of guaiac at 3.3 but not at 3.2. Tyrosin was not oxidized at any pH value under the conditions of the tests, i.e. on addition of a dilute solution of the indicator to the buffered sample

¹ We have followed the usual practice of considering a plant oxidase to consist of an organic peroxide and a peroxidase.

of juice. The optimum pH value varied with the different indicators. Allowing for atmospheric oxidation at high pH values, the optimum pH value for the oxidation of pyrogallol appeared to be at about 7; hydroquinon about 8–8.5; para-cresol and the ortho-and para-amino-phenols about 5.5–6; para-phenylene-diamin-hydrochloride at about pH 5 and guaiacol about 5.0 to 5.5. The necessary color comparisons were made with a Klett colorimeter. Because of the rapid fading of the oxidized color of tincture of guaiac it was difficult to establish its optimum pH, although it apparently colored by oxidation most rapidly at pH 5.0 to 8.0.

On the alkaline side of neutrality the darkening of the various indicators was inhibited at different pH values. Guaiacol failed to oxidize at pH 9.5, benzidine at 9.8, α -naphthol at about 10.0 and tincture of guaiac at 9.8; para-cresol and para-phenylene-diamin-hydrochloride between 9.2 and 11.5. The other indicators showed marked atmospheric oxidation at pH values above 7.8 and it was therefore difficult to establish their upper pH limits although above pH 9.2 the intensity of combined atmospheric and oxidasic oxidation rapidly decreased with increase of pH value.

It was apparent that the choice of indicator affected the pH maximum, minimum and optimum for apricot oxidase. Similar results were obtained with the peroxidase of peaches and pears. In general, oxidase action on all of the indicators was much less at low pH values than at those near neutrality, and decreased with decrease in pH value to a vanishing point between pH 2 and pH 3 for most of the indicators tested.

Effect of H_2O_2 concentration

BERNHEIM and DIXON (1) and WILLSTÄTTER (8) as well as others have reported that peroxidase activity is affected by the concentration of added H_2O_2 . We found that the peroxidase of the apricot is similarly affected although the optimum and the maximum values varied somewhat with the indicator. By addition of the H_2O_2 and indicator to juice from cold stored apricots and peaches (very low in catalase activity) the optimum range for hydroquinon was found to be from 15–25 mg. per 100 cc.; for α -naphthol 5–10 mg.; pyrogallol, 5–10 mg.; and for guaiacol, ortho-amino-phenol and para-cresol approximately 10 mg. per 100 cc. At 480 mg. of H_2O_2 per 100 cc., the peroxidase reactions with α -naphthol and hydroquinon were negative and at 360 mg. were negative for pyrogallol. The optimum is naturally affected by the relative activity of the catalase which tends to rapidly destroy the H_2O_2 in juices rich in catalase. The catalase activity is affected by the pH value of the medium [see OVERHOLSER (4)]. The pH value of the apricot juice was approximately 4.0; that of the peach juice slightly less than 4.

Inactivation pH values for peroxidase

Apricot juice was brought by addition of tartaric acid, or sodium hydroxide and sodium bicarbonate to pH values ranging from 1.75 to 13.0. At intervals small portions of each sample were brought to approximately pH 7.0 and were tested with several peroxidase indicators. On the acid side of neutrality the peroxidase was apparently destroyed in 3 hours or less between pH 2.0 and 2.1, and on the alkaline side at about pH 12.8. At 18 hours the peroxidase was destroyed at about pH 12.1 and at 48 hours at about 11.7. The organic peroxide was at least temporarily utilized or inactivated at pH 8.5.

As previously reported (3) the temperature also affects the pH value necessary for inactivation; or rather conversely, the temperature necessary for inactivation varies with the pH value; the oxidase being most resistant to high temperatures near neutrality. Our earlier results were confirmed by quantitative colorimetric measurements using several oxidase indicators; the effect of heat at various pH values was found to be progressive rather than abrupt. These determinations were made both with the natural juice and with the peroxidase prepared by precipitation with ethyl alcohol and reprecipitation with acetone.

Summary

1. The optimum, minimum and maximum pH values for various oxidase indicators in contact with apricot oxidase vary with the indicator; but in general the optimum for most of the indicators used in these studies lies near neutrality.

2. The hydrogen peroxide concentration is not a matter of indifference, but greatly affects the intensity of the oxidase reaction with various indicators, the optimum range of H_2O_2 concentration in apricot juice low in catalase being from 5 to 25 mg. of H_2O_2 per 100 cc.

3. The peroxidase was inactivated at about pH 2.0 and at about 12.8 in less than 3 hours and on subsequent adjustment to neutrality failed to respond positively with the usual peroxidase indicators. The organic peroxide was considerably less resistant than the peroxidase to high pH values.

4. In general, in any comparative studies on the activity of fruit oxidase the pH value of the medium and the H_2O_2 concentration must be carefully controlled and the proper indicator chosen.

FRUIT PRODUCTS LABORATORY,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. BERNHEIM, F., and DIXON, M. Studies on xanthine oxidase. X. The action of light. *Biochem. Jour.* **22**: 113-124. 1928.

2. CLARK, W. M., and ZOLLER, H. F. A series of oxidation-reduction indicators. *Science* **54**: 557. 1921. See also, CLARK, W. M. Recent studies on reversible oxidation-reduction in organic systems. *Chem. Rev.* **2**: 127-178. 1925.
3. CRUESS, W. V., and FONG, W. Y. Observations on fruit oxidase. *Fruit Products Jour.* **6**: 13-15. 1926.
4. OVERHOLSER, E. L. A study of the catalase of the fruits of pear varieties. *Amer. Jour. Bot.* **15**: 285-306. 1928.
5. —————, and CRUESS, W. V. A study of the darkening of apple tissue. *Agr. Exp. Sta. Univ. of California Tech. Bull.* **7**. 1923.
6. RAPER, H. S. Die Einwirkung von Tyrosinase auf Tyrosin. *Fermentforschung* **9**: 206-213. 1927.
7. UCKO, H., and BANSI, H. W. Über Peroxydase. IV. Die Bedeutung des Substrates für das pH-Optimum. *Zeitschr. physiol. Chem.* **164**: 52-57. 1927.
8. WILLSTÄTTER, R., and WEBER, HANS. Über Peroxydase. V. Zur quantitativen Bestimmung der Peroxydase. *Liebig's Ann.* **449**: 156-174. 1926. See also, VI. Hemmung der Peroxydase durch Hydroperoxid. **449**: 175-187. 1926.

TESTING THE SUGAR CONTENT OF BEETS FOR GENETICAL PURPOSES

ERNEST REED

Introduction

Since sugar beets are raised solely for sugar the one characteristic of prime importance is the sugar content. It has been shown (6) that some of the difficulties involved in the breeding of beets are matters of flower development and fertilization. PRITCHARD (5) claimed that there is no correlation between morphological characters and sugar content and further that sugar content of the beet is not inherited. Such studies are dependent upon exact control of pollination and a correct determination of sugar content in the beet. The purpose of this paper is to point out a source of error in the methods¹ now used in sugar beet testing and to present a method in which this particular error will not be possible.

There are various methods in use for sampling individual beets for sugar determinations (1, 2, 3, 4, 7, 8, 9). The simpler of these methods is to grind the beet pulp and extract the juice. Another method involves boring a hole through the beet and catching the pulp and juice as they ooze out. The underlying principle of these methods is to break the cells of the beet tissue. Lead sub-acetate is then added to the normal weight of the pulp and the sugar diffuses throughout the liquid because of the cells having been broken. Ten or more minutes are allowed for cold water digestion.

Theoretically, polarization of the filtrate from these preparations should give a relatively accurate reading of the percentage of sugar content in the beet. Actually, however, mechanical devices which it is necessary to use either in grinding or boring the beet cannot be relied upon to break all of the cells. Since hundreds, and often thousands, of beets are tested in this way the test for the group may vary in proportion to the efficiency of the grinding machine or the boring rasp which may be used in securing the pulp. The rasp may be in perfect cutting condition for the first beet but it naturally becomes duller as subsequent borings are made so that a variation in sugar content which is due to the method will make the data valueless.

Experimental

In connection with the work reported in this paper, six samples of pulp were secured from the same beet with a newly sharpened rasp made specifically for this purpose. Cold water digestion for thirty minutes was

¹ This work is concerned only with simple and rapid methods which may be used in the testing of several hundred beets a day by one or two persons.

allowed before filtration and the six samples were treated alike and polarized at the same time with the same polariscope. The six samples tested as follows:

1st	6.2	per cent.
2nd	6.7	“ “
3rd	6.2	“ “
4th	6.7	“ “
5th	7.5	“ “
6th	8.7	“ “

We have here a range of from 6.2 per cent. to 8.7 per cent. in six tests from one beet, a variation of 2.5 per cent. sugar content or a possible error of 24.8 per cent. in the calculation. Some of the remaining pulp from these samples was examined with the microscope and it was found that quite a few of the cells were not broken. Similar tests were made from many other beets and a wide range of sugar content was secured for every beet thus sampled several times.

The essential thing in the cold water or warm water digestion methods is that every cell should be so broken down that the sugar from within may freely diffuse and so be represented in the filtrate which is polarized. The mechanical devices which are used in testing large numbers of beets which are necessary in beet breeding studies must of necessity introduce a large error, so large in fact that the data become useless for genetical analysis.

Twelve more samples were secured from the same beet mentioned above. Six of these samples were secured by the boring rasp and six others were secured as chunks cut out with a knife. The necessary lead sub-acetate water was added to each sample and all were placed in an autoclave. Here they were exposed to 15 pounds pressure for 15 minutes and then allowed to cool to room temperature. The filtrates from these samples were polarized and each gave the same reading, a 10 per cent. sugar content value.

In this study is involved ground pulp in six of the samples, and chunks of beet tissue in the other six. There are probably large numbers of unbroken cells in the pulp and the cells of the chunks are not broken except along the outside surfaces. The autoclave treatment breaks down the otherwise sugar impermeableness of the cells and so makes possible free diffusion of the sugar from all of the cells. If this is true the tests of each sample should be the same as that of any other sample and we see that this is the case.

But we also find that each sample that has gone through the autoclave gives a sugar percentage reading which is higher than the highest test from those samples which were not put through the autoclave. There is a difference of 1.3 per cent. between the autoclave sugar reading and the highest

non-autoclave sugar reading and the greatest difference between the autoclave and the non-autoclave samples is 3.8 per cent.

Subsequent studies, on many other beets, were made similar to the above, with the same fundamental differences occurring between the two methods, and also with all of the samples from any one beet testing the same when put through the autoclave, whether as pulp or as chunks.

The question which naturally arises is this. Does not the autoclave method so affect the carbohydrate contents of the beet sample as to make available more material which might be recorded on the polariscope along with and in addition to the sucrose?

A known amount of sugar (cube sugar) was added to certain samples and some of these were subjected only to the cold water digestion and others to the autoclave. There resulted no difference, which indicated that the autoclave did not affect the ordinary sugar which was added to the lead sub-acetate. This, however, did not provide against possible changes which might occur on carbohydrates in the beet tissue.

Finally the following method was used and seems to serve as a convincing check on the autoclave method.

Thirty samples were taken from the one beet and the correct amount of lead water added to each. Fifteen of these samples were put through the autoclave and the resulting sugar test was 15.8 per cent. in each case. The other fifteen samples were frozen at -80° C. by the use of solid carbon dioxide. Here again, the test in each case was 15.8 per cent.

It is to be expected that the freezing method would slow down chemical reaction in the beet tissue to a minimum. The only changes occurring in the beet tissues thus frozen would be physical changes, such as might destroy the sugar impermeableness of the cells. The freezing method was repeated with twenty other beets and in each case an equal number of tests was made from each beet with the non-autoclave method and the autoclave method. The results were always the same, namely, the non-autoclave method gave a lower test than the other two methods. The autoclave and freezing tests checked and further the tests secured by the non-autoclave method for any one beet did not agree with each other, whereas the autoclave and the freezing method tests for any one beet were always the same.

Discussion

It has been pointed out by PRITCHARD (5) and others, that the data which have been secured from extensive studies in sugar beet breeding show no apparent correlation between any of the characteristics of the beet and the sugar content. There is a large amount of data at hand which indicates that sugar content is not a heritable characteristic. These interpretations of the data are not only justified but seem to be the only conclusions that it is possible to draw from the data.

There are two possible sources of error; that involved in fertilization, Reed (6), and that error which is due to the methods of pulp extraction for sugar testing, as explained in this paper.

When beets are tested for sugar content it is assumed, and rightly so, that when the cells of the beet are broken in water the sucrose will diffuse throughout the water. This water may then be polarized and the sugar percentage thus determined. Lead sub-acetate is used with the water as a clarifying agent.

It becomes apparent that the accuracy of the sugar test depends primarily upon the efficiency of the instrument which is to break down the cells. Any cells which are not broken will not only retain their sugar but will also take up water. There have been devised various rasps for boring through the beet. These rasps are so provided with cutting teeth that they are expected to cut every cell of the beet pulp. This pulp is not further treated in order to insure against any of the cells not being broken but it is weighed out and subjected to cold water digestion. Any unbroken cells are not detected and the extent to which unbroken cells may occur in this pulp will be registered in the percentage reading as an error.

This possibility of error was demonstrated to be very great by the microscopical examination of pulp which was secured by the use of the rasp. The occurrence of this error was definitely demonstrated by the differences between the tests which were made on several samples from the same beet.

With this source of error in mind, the accumulated data, which have been used to draw the conclusions that sugar content is not inherited, and that there is no correlation between phenotypic characteristics and sugar content, become useless and the conclusions are of no value. Knowing the extent of error which is likely to be involved in such work, it is to be expected that sugar percentages secured by the use of these methods shall vary greatly and be of no use for genetical analyses.

By use of the autoclave, as described in this paper, one may secure accurate sugar percentage readings of individual beets. The method is simple, the operations are more easily and more quickly performed, as it is neither necessary to grind the beet tissue nor to bore it. The theory underlying this method is the same as that of the other methods but in this case the sugar permeableness of the cells is guaranteed by the effectiveness of the autoclave treatment.

The accuracy of the autoclave method is demonstrated by the fact that the several samples taken from the same beet test the same when this method is used, and by the fact that the tests which are secured by use of the autoclave method check with those of the freezing method. The data presented earlier in this paper show that the total sugar is not recorded.

in any sample secured by use of the rasp, whereas it is apparent that the autoclave method insures a test of all of the sugar of the sample. This statement is substantiated by the fact that the tests by the use of the autoclave method agree with those of the freezing method.

By using solid carbon dioxide to freeze the tissue, we have a reliable check on the autoclave method. Some such check is necessary because our knowledge of the changes which may take place in beet tissue in the autoclave is not sufficient.

Summary

The following method is recommended for determining the sugar content of individual beets where such is desired for genetical study, where accuracy is essential.

The normal weight (26.048 grams) of the beet pulp is put into a lead subacetate solution (thirty baume) 177 cc. This is autoclaved at 15 pounds for 15 minutes and allowed to cool to room temperature. The solution is then filtered and the filtrate polarized.

DEPARTMENT OF BOTANY,
SYRACUSE UNIVERSITY.

LITERATURE CITED

1. BEAUDET, L., PELLET, H., and SAILLARD, C. *Traité de la fabrication du sucre de betterave et de canne.* t. 2. Paris. 1894.
2. CLARK, W. B. A sampling press. *Jour. Ind. Eng. Chem.* **9**: 788-790. 1917.
3. PACK, D. A. Disc sampling machine. *Sugar (New York)* **26**: 97. 1924.
4. PELLET, H. Dosage du sucre cristallisable dans la betterave. *Ann. Sci. Agron. Franç. et Étrang.* **9** (1): 316-473. 1892.
5. PRITCHARD, FREDERICK J. Some recent investigations in sugar beet breeding. *Bot. Gaz.* **62**: 425-465. 1916.
6. REED, ERNEST. Sterility and inbreeding in beets. *Memoirs Hort. Soc. New York.* **3**: 59-63. 1927.
7. SAILLARD, E. *Betterave et sucrerie de betteraves.* pp. 618. Paris. 1913.
8. SHERWOOD, S. F. Sucrose in seed beets. *Sugar (New York)* **23**: 299-300. 1921.
9. ————. Use of the refractometer in the analysis of individual sugar beets. *Jour. Agr. Res.* **36**: 41-52. 1928.

EFFECT OF ACID AND ALKALINE HYDROLYSIS ON THE ESTIMATION OF HEMICELLULOSE AND ASSO- CIATED GROUPS IN YOUNG APPLE WOOD

FISK GERHARDT

The greater portion of the food reserve and structural network in plants is composed of carbohydrate material. In the vegetative tissue and seed of many plants, there exists a group of hexosan-pentosan polysaccharides to which SCHULZE has given the name of "hemicelluloses." These hemicelluloses are easily hydrolyzed by dilute acids into simple hexose and pentose sugars or may be isolated from the tissue in the colloidal form by means of weak caustic solutions. The characterization of this group of substances is even less specific than our present definition of cellulose. This apparent indefiniteness of classification is due partially to the fact that neither of the above methods of isolation effects a sharp separation of cellulose from lignin and the associated hemicellulose groupings.

As a means of orientation in the general problem of investigating the distribution and function of these polyhexose-pentose structures in the metabolism of the plant, it became of interest to study the two usual methods of their determination, and further to note the effect of such procedures upon the tissue components associated with them. An attempt was also made to identify the sugars present in the hemicellulose fraction.

No effort will be made here to review the voluminous literature pertaining to the distribution of these anhydride sugars in plants. The earlier work of SCHULZE (27, 28) has been enlarged through the efforts of O'DWYER (22, 23, 24), CANDLIN and SCHRYVER (3), NORRIS and SCHRYVER (21), and LING and NANJI (15) in a series of papers dealing with the isolation, properties and constitution of the hemicelluloses. KARRER (13) found a reserve cellulose (lichenin) present in lichens which yielded only glucose upon hydrolysis. O'DWYER (23) and EHRLICH (7) have shown that hemicelluloses are not true carbohydrates (pentosans, hexosans), but also contain acid groups of the glucuronic type and are more nearly like the pectins than like true cellulose. VON FELLEBERG (33) EHRLICH (7) and CANDLIN (3) suggest the possibility that hemicelluloses may prove to be the intermediate stage between pectin and lignin because of the fact that unignified tissues have a larger proportion of pectins than hemicellulose, while in the lignified material the opposite is true. The transition of pectin into hemicellulose is presumed to occur through a process of decarboxylation (24).

In the resolution of the hemicelluloses during acid or alkaline hydrolysis certain changes are produced in the residual tissues. These changes may alter the composition of the hemicellulose fraction. NORRIS and SCHRYVER

(21) have shown that the method of pectin removal influences the composition of hemicellulose, while TOTTINGHAM and GERHARDT (32) and O'DWYER (23) found that the kind, strength, and duration of hydrolysis also exerts a specific effect. According to SHERRARD (30) weak acid hydrolysis destroys over 30 per cent. of the cellulose, 83 per cent. of the pentosans, and over 16 per cent. of the methoxy-groups in wood. PETERSON and HIXON (25) have shown that the alkaline extraction of the hemicellulose in the cornstalk also removes large amounts of lignin. HAWLEY and CAMPBELL (11) state that partial hydrolysis effects definite changes in wood similar to those produced by certain white and brown-rot fungi (10). These changes involve a destruction of the pentosan and cellulose fractions, and increase the alkali solubility (lignin) of the wood.

Material and methods

One year old apple wood was used as the experimental material. This was collected as one large sample (one kilo dry matter) on April 10, 1928. After removal to the laboratory the tissues were cut into small pieces and desiccated under reduced pressure at 70° C. The material was then ground to pass a 60 mesh sieve. The air-dried wood contained 4.75 per cent. moisture and 3.47 per cent. ether soluble material.

Lignin and cellulose were determined by treatment of the tissue with 4 per cent. NaOH at 180 pounds pressure for one hour according to the method of MEHTA (16).

Methoxy-groups were identified by the ZEISEL method as modified by DORE (6).

Pentosan determinations were made by distillation with 12 per cent. HCl according to the Official Methods (1).

"Uronic" acids were determined by precipitation of the liberated carbon dioxide with Ba(OH)₂ as specified by NANJI, PATTEN and LING (19).

Pectins were removed by extraction of the tissue with 0.50 per cent. (NH₄)₂C₂O₄ according to CLAYSON, NORRIS and SCHRYVER (4).

Dilute acid hydrolysis was accomplished by boiling the residual tissue after pectin removal with 2.50 per cent. H₂SO₄ (by weight) for 2 hours.

Dilute alkaline extraction of the hemicellulose fraction from the tissue after pectin removal was accomplished by treatment with 4 per cent. NaOH at room temperature for 24 hours (23).

The hot water extract represents that fraction of the tissue removed by boiling during the enzymatic digestion of the starch.

Experimental

COMPOSITION OF THE TISSUE AS AFFECTED BY PRELIMINARY TREATMENT

DORE (5) has shown that partial hydrolysis affects the more residual components of the plant cell, especially the methoxy-content of the lignin

and the furfural yielding constituents of the cellulose. In the following procedure, young apple wood was subjected to certain extractive and hydrolytic treatments. Analyses were made of the untreated and the residual tissues, and a comparison of these data gives an indication of the specific effect of each procedure.

The percentage of tissue removed during the different specific treatments was determined as follows: four aliquot samples were freed from hot water-soluble constituents including starch, and dried to a constant weight. Two samples were then used for analysis of the tissue, while the remaining two were further extracted with ammonium oxalate for removal of pectin, and after desiccation to constant weight, were again subjected to analysis. The acid and alkaline treatments for hemicellulose removal were confined to samples which had previously been freed from water-soluble, starch, and pectic materials. The percentage of tissue accounted for represents the summation of the hot-water extract, ammonium oxalate extract, loss on dilute acid hydrolysis, pentosan-free cellulose, pentosan in cellulose, pentosan destroyed during the cellulose determination, lignin and ether extract. These data are assembled in table I.

The data in table I indicate that preliminary treatment produces a marked change in the approximate analysis of the wood, hot water alone removing 6 per cent. of the pentosan and sugar acids of the glucuronic type. Removal of the pectic constituents with $(\text{NH}_4)_2\text{C}_2\text{O}_4$ produces little change in cellulose, lignin, or methoxy-content. However, about one half of the total "uronic" acids and 20 per cent. of the total pentosans in normal apple wood are associated with pectic compounds, the structural formula of pectin as suggested by NANJİ (19) presupposing the presence of galactose, pentoses and sugar acids.

Decided differences in composition of the residual tissue are produced by hemicellulose removal by either acid or alkaline hydrolysis. Both methods remove approximately the same amount of total material but their specific effect upon the residual tissues is quite different. Acid treatment reduces the yield of cellulose by almost 30 per cent. and reduces the residual pentosan therein by over three-fourths. Its action upon lignin is largely confined to a hydrolysis of its methoxy-groups, since the loss in methoxy and total lignin approach a common value. Approximately one-half of the total pentosans in the tissue is removed, while the "uronic" acids are affected but little. The latter fact is an indication that about one-half of the CO_2 liberated and calculated as "uronic" acids has its origin in the pectic constituents, while the greater portion of the remainder is found, not in the hemicellulose fraction, but rather associated with the more residual skeletal components. The loss in cellulose due to acid treatment is largely

TABLE I

A. PERCENTAGE COMPOSITION OF 1 YEAR OLD APPLE WOOD BEFORE AND AFTER VARIOUS SPECIFIC TREATMENTS (MOISTURE-FREE BASIS)

SPECIFIC TREATMENT	CELLULOSE	LIGNIN	PENTOSANS IN CELLULOSE CALCULATED TO ORIGINAL WOOD	TOTAL PENTOSANS IN TISSUE	METHOXY ACIDS	PERCENTAGE OF TISSUE REMOVED	PERCENTAGE OF TISSUE RECOVERED
Normal wood, un- treated	25.96	12.50	2.70	19.46	3.60	0.00	98.11
Extracted with hot water (starch free) .	25.86	12.00	2.70	18.12	3.57	23.40	97.96
Extracted with ammo- nium oxalate	25.32	12.00	2.63	15.44	3.30	7.60	97.79
Hemicellulose removed by acid hydrolysis	18.76	10.88	0.65	7.33	3.07	21.00	91.79
Hemicellulose removed by alkaline hydro- lysis	23.40	6.86	1.54	6.86	2.85	18.47	88.50

B. PERCENTAGE LOSS OF TISSUE COMPONENTS AFTER VARIOUS SPECIFIC TREATMENTS

Extraction with water	0.38	0.41	0.00	6.88	0.88	6.77	
Extraction with ammo- nium oxalate	2.46	0.41	1.51	20.65	7.81	47.26	
Hydrolysis with acid	27.70	9.70	75.90	52.66	8.17	9.60	
Hydrolysis with alkali	9.86	43.07	42.90	55.50	13.63	12.80	

accounted for by an increase in the beta and gamma forms (16) in the liquors from the cellulose determination. It is interesting to note that even such a drastic acid preliminary treatment does not remove completely the residual pentosan complex from the cellulose. The furfural value derived from the cellulose is due to pentosans and not to oxycelluloses because no characteristic color reactions or decarboxylation of the cellulose, indicating the presence of such groups, could be obtained.

Resolution of the hemicellulose by alkali produces no great destructive action on the cellulose residue. However, at room temperatures it removes almost one-half of the total lignin of the wood. In this case the methoxy content is not destroyed in proportion to the total lignin. The lignin must be removed either as a straight solubility product or by hydrolysis and removal as the soluble sodium salt of lignic acid. Weak alkali removes approximately 3 per cent. less tissue than does acid hydrolysis. The percentage recovery of total tissue is also lower in the alkaline extracted wood.

CARBOHYDRATE RECOVERY IN THE ACID AND ALKALINE HYDROLYZATES

During the usual carbohydrate analysis of plant tissue, the hemicellulose fraction is estimated by a direct weak acid hydrolysis. Since acid and alkali remove almost like amounts of total tissue, it became of interest to determine whether the reducing sugar values and furfural yields also bore a similar relationship.

Two 25-gm. samples of apple wood were freed from starch and water-soluble materials. Pectin was removed by 0.50 per cent. $(\text{NH}_4)_2\text{C}_2\text{O}_4$. One such residue was refluxed on an electric hot plate with 2.50 per cent. H_2SO_4 for a period of 2 hours. The other residue was extracted with 4 per cent. NaOH at room temperature in a shaking machine. The filtered alkaline extract was neutralized and brought to an acid concentration of 2.5 per cent. H_2SO_4 and refluxed for 2 hours. The clarified hydrolyzates from both pro-

TABLE II
CARBOHYDRATE RECOVERY IN THE HEMICELLULOSE OF YOUNG APPLE WOOD

TREATMENT APPLIED TO TISSUE	REDUCING POWER IN EQUIVALENTS OF GLUCOSE	PENTOSAN	"URONIC" ACIDS	TOTAL TISSUE REMOVED
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Acid hydrolysis	10.61	10.76	0.21	21.00
Alkaline extrac- tion	7.20	8.40	0.19	18.60

cedures were brought to volume and their composition based upon the original moisture-free wood is shown in table II.

Although dilute acid removes 21 per cent. of the original tissue, only approximately one half of this amount can be recovered in the form of sugar as shown in table II. The similarity of reducing sugar and pentosan values indicates that the hemicellulose fraction of young apple wood is polypentose in nature. TOTTINGHAM (31) has reported the presence of polyhexosans in the base wood of the apple. His technique, however, did not include the removal of the pectic constituents prior to acid hydrolysis. The amount of "uronic acids" in the products of hydrolysis is exceedingly small, over one half of the total amount present in the wood having been removed with the pectic material in the ammonium oxalate extract.

The reducing power and pentosan value of the alkaline hemicellulose extract are considerably lower than in the acid hydrolyzate. Either the 4 per cent. NaOH destroys some of the carbohydrate material, or its action upon the tissue is less drastic. When the tissue residue from the 4 per cent. NaOH extraction was further treated with boiling 2.5 per cent. H_2SO_4 for 2 hours, the hydrolyzate contained an additional reducing power of 4.60 per cent. and a pentosan value of 4.10 per cent. The carbohydrates from this second hydrolysis are also derivatives of polypentose material and imply a similar origin in the tissue. These results indicate that alkaline extraction liberates less of the hemicellulose fraction from the tissue than does direct acid hydrolysis. WINTERSTEIN (34) however, has stated that 5 per cent. alkali probably modifies the residual cellulose so that it will again respond to subsequent dilute acid hydrolysis.

ISOLATION AND ANALYSIS OF THE HEMICELLULOSE FROM YOUNG APPLE WOOD

After removing the starch with a saliva digestion, 200 gm. of the tissue were extracted with 1 liter of 0.5 per cent. $(\text{NH}_4)_2\text{C}_2\text{O}_4$ at 85°C . for 24 hours to insure complete removal of pectic materials. Proteins, coloring matter, etc., were removed with 1 per cent. NH_4OH . The residue from the NH_4OH extract was further treated with 1 liter of 4 per cent. NaOH at room temperature for 24 hours. The filtered alkaline extract was neutralized with acetic acid and the hemicellulose precipitated by the addition of an equal volume of 95 per cent. alcohol. After washing thoroughly with 60 per cent. alcohol, the precipitate was again brought into solution in 1 per cent. NaOH, reprecipitated with acetic acid and alcohol. It was washed with gradient strengths of alcohol and ether and finally dried overnight at 100°C . A yield of 17.2 gm. (8.60 per cent.) of a light gray amorphous material was obtained. It had the following composition:

	Per cent.
Moisture	5.78
Reducing sugar	69.00
Lignin	14.28
Ash	5.19
Total	94.25

The furfural yield of this substance in equivalents of pentosan, amounted to 68.5 per cent. The product was laevorotatory $[\alpha]^{25}_D = -104^\circ$ in 1 per cent. NaOH. The reducing power and pentosan value again indicate its polypentose nature.

HYDROLYSIS AND IDENTIFICATION OF THE SUGARS IN THE HEMICELLULOSE FRACTION

Three 2-gm. samples of the purified hemicellulose were hydrolyzed with 2.5 per cent. H_2SO_4 for a period of 3 hours. In each case after hydrolysis there remained an insoluble residue which amounted to 5.60 per cent. of the original sample. The three hydrolyzates were digested with $Ba(OH)_2$, clarified with neutral $Pb(C_2H_3O_2)_2$ and concentrated under reduced pressure to a volume of 25 cc. and a concentration of 7.20 per cent. The three hydrolyzates, using a 200-mm. tube, gave an average reading of $+13^\circ$ on the Ventzke sugar scale, or a specific rotation $[\alpha]^{25}_D = +31.3^\circ$. Aliquot portions had a reducing power in equivalents of glucose of 69.00 per cent.

In view of the fact that the reducing sugar and pentosan values are of approximately the same magnitude, it appears probable that pentose sugars alone are present. This situation is further substantiated by the failure to identify the presence of galactose through oxidation to mucic acid (14) or of mannose by its insoluble phenylhydrazone (8), or of ketohexoses by the diphenylamine (12) PINOFF (26) or SELIWANOFF (29) test for fructose, or of glucose through oxidation to saccharic acid (9). These results are strong evidence that hexosans are not present in this material in significant amounts.

Five grams of the purified amorphous grey hemicellulose substance were again hydrolyzed, de-sulphated and clarified as previously described. The filtrate was concentrated under partial *vacuo* and purified according to the method of MORROW (17). Under no circumstances, however, was it possible to effect crystallization of the sugars either from alcohol or glacial acetic acid. An aliquot portion of the syrup was treated with 1 gm. of phenylhydrazine hydrochloride and 1.5 gm. of sodium acetate in a test-tube in a boiling water bath according to MULLIKEN (18). An osazone in the form of an orange-yellow mass appeared after 8–10 minutes' heating. The surface of the solution also contained oily drops. Microscopical observa-

tion and melting-point determinations identified the recrystallized product as the osazone of xylose. This sugar was further identified by oxidation with bromine and the formation of the characteristic crystals of the double cadmium salt of xylose. As oily drops in the osazone reactions are an indication of arabinose, an attempt was made to obtain its diphenylhydrazone by treating another aliquot of the syrup with an excess of diphenylhydrazine according to NEUBERG (20). After cooling the solution, a white crystalline hydrazone appeared. It melted at 207° C. and must represent the hydrazone of arabinose.

Taking MORROW's (17) value for the specific rotatory power of d-xylose, namely $[\alpha]^{25}_D = +19^\circ$ and that of l-arabinose as $[\alpha]^{25}_D = +104$, a mixture of 7 mols. of xylose with 1 mol. of arabinose would have a specific rotation $[\alpha]^{25}_D = +29.6^\circ$ a number in fair agreement with the mean observed value of $[\alpha]^{25}_D = +31.3^\circ$ for the sugar mixture in the hemicellulose fraction. Furthermore, 8 mols. of such a mixture having an empirical formula of $C_5H_{10}O_5$, should contain 7 mols. of d-xylose or 87.5 per cent., and 1 mol. of l-arabinose or 12.5 per cent. According to BROWN (2) the percentage of sugars in a mixture may be calculated by combining the polariscopic and reducing sugar values in a simultaneous equation. Calculated by this method, the sugars in the hemicellulose fraction were found to be composed of 88.4 per cent. d-xylose and 12.7 per cent. l-arabinose,—figures in close agreement with the theoretical value. An attempt was made to quantitatively determine the arabinose present in the syrup according to NEUBERG (20). 10 cc. of the syrup corresponding to 0.720 gm. of the original hemicellulose or 0.497 gm. of sugar therein, when treated with a slight excess of diphenylhydrazone, yielded 0.144 gm. of arabinose diphenylhydrazone or 0.068 gm. arabinose which is equal to 13.70 per cent. of the total sugar present. Although both the observed and calculated values do not agree exactly with theory, they serve to indicate the molal ratio in which the two sugars are probably present.

Summary

A study has been made of the action of several extractive and hydrolytic procedures upon young apple wood during the isolation of the hemicellulose fraction. Hot water and salivary digestion removed 23 per cent of the tissue. Its action upon the residual tissue was slight. Ammonium oxalate removed 7 per cent. (largely pectic constituents) of the tissue. About one-fourth of the pentosans and one-half of the "uronic" acids were removed in this fraction. Dilute acid hydrolysis removed 21 per cent. of the ammonium oxalate extracted residue. It also removed 10 per cent. of the lignin, 50 per cent. of the remaining pentosans and altered 30 per cent. of the cellulose. 4 per cent. NaOH at room temperature removed less car-

bohydrate and total tissue than did the acid treatment. It, however, removed almost one-half of the total lignin.

The isolated hemicellulose fraction of a 1-year-old apple wood has been purified and analyzed. It contained approximately 15 per cent. lignin, 70 per cent. pentose sugars, 5 per cent. ash, and 5 per cent. moisture. No hexoses were identified therein, d-xylose and l-arabinose were present, probably in a molal ratio of 7 to 1 respectively. 88 per cent. of the total reducing sugar present in this fraction was d-xylose and the remaining 12 per cent. was found to be l-arabinose.

CHEMISTRY SECTION,
IOWA AGRICULTURAL EXPERIMENT STATION.

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 2nd ed. Washington, D. C. 1925.
2. BROWN, S. A. Handbook of sugar analysis. 1st ed. John Wiley & Sons. 1912.
3. CANDLIN, E. J., and SCHRYVER, S. B. Investigations of the cell-wall substances of plants with special reference to the chemical changes taking place during lignification. Proc. Roy. Soc. London B. **103**: 365-376. 1928.
4. CLAYSON, D. E. F., NORRIS, F. W., and SCHRYVER, S. B. The pectic substances of plants. Part II. A preliminary investigation of the chemistry of the cell-walls of plants. Biochem. Jour. **15**: 643-653. 1921.
5. DORE, W. H. The determination of cellulose in woods. Jour. Ind. Eng. Chem. **12**: 264-269. 1920.
6. ————. The distribution of certain chemical constants of wood over its proximate constituents. Jour. Ind. Eng. Chem. **12**: 472-476. 1920.
7. EHRLICH, F. Neue Untersuchungen über Pektinstoffe. Zeitschr. angew. Chem. **40**: 1305-1313. 1927.
8. FISCHER, E., and HIRSCHBERGER, J. Über Mannose. Ber. deutsch. chem. Ges. **21**: 1805-1809. 1888. See also **22**: 365-376. 1889.
9. GANS, R., and TOLLENS, B. Über die Bildung von Zuckersäure als Reaction auf Dextrose.—Raffinose enthält Dextrose. Ann. Chem. **249**: 215-227. 1888.
10. HAWLEY, L. F., FLECK, L. C., and RICHARDS, C. A. Effect of decay on the chemical composition of wood. Jour. Ind. Eng. Chem. **20**: 504-507. 1928.
11. ————, and CAMPBELL, W. G. Effect of partial hydrolysis on the alkali-solubility of wood. Jour. Ind. Eng. Chem. **19**: 742-744. 1927.

12. IHL, A. Über die Einwirkung von Diphenylamine auf Kohlenhydrate bei Gegenwart von Alcohol, Schwefelsäure oder Salzsäure. *Chem. Zeitg.* **9**: 451. 1885.
13. KARRER, P. Über Reservecellulose und Cellulose. *Zeitschr. angew. Chem.* **37**: 1003–1007. 1924.
14. KENT, W. H., and TOLLENS, B. Untersuchungen über Milchzucker und Galactose. *Ann. Chem.* **227**: 221–232. 1885.
15. LING, A. R., and NANJI, D. R. The nature of the amylo-hemicellulose constituent of certain starches. *Jour. Chem. Soc.* **127**: 652–656. 1925.
16. MEHTA, M. M. The nature of lignin: Its physiological significance and its estimation in timbers. *Biochem. Jour.* **19**: 958–978. 1925.
17. MORROW, C. A. *Biochemical laboratory methods.* John Wiley & Sons. 1st ed. 1927.
18. MULLIKEN, S. P. A method for the identification of pure organic compounds. John Wiley & Sons. 1st ed. 1905.
19. NANJI, D. R., PATON, F. J., and LING, A. R. Decarboxylation of polysaccharide acids: Its application to the establishment of the constitution of pectins and to their determination. *Jour. Soc. Chem. Ind. (Trans.)* **44**: 253T–258T. 1925.
20. NEUBERG, C. Über die Harmpentose, ein optisch inactives, natürlich vorkommendes Kohlehydrat. *Ber. deutsch. chem. Ges.* **33**: 2243–2254. 1900.
21. NORRIS, F. W., and SCHRYVER, S. B. The nature of pectinogen and its relationship to pectic acid. *Biochem. Jour.* **19**: 676–693. 1925.
22. O'DWYER, MARGARET H. The hemicelluloses. III. The hemicellulose of American white oak. *Biochem. Jour.* **17**: 501–509. 1923.
23. ————. The hemicelluloses of beech wood. *Biochem. Jour.* **20**: 656–664. 1926.
24. ————. Preliminary investigations on the constitution of the hemicelluloses of timber. *Biochem. Jour.* **22**: 381–390. 1928.
25. PETERSON, C. J., and HIXON, R. M. Chemical examination of the tissue of the cornstalk. *Jour. Ind. Eng. Chem. (analytical ed.)* **1**: 65–67. 1929.
26. PINOFF, E. Über einige Farben- und Spectral-Reactionen der wichtigsten Zuckerarten. *Ber. deutsch. chem. Ges.* **38**: 3308–3318. 1905.
27. SCHULZE, E. Zur Kenntniss der chemischen Zusammensetzung der Pflanzenzellmembranen. *Ber. deutsch. chem. Ges.* **22**: 1192–1196. 1889. **23**: 2579–2583. 1890. (See also **27**: 3525–3527. 1894. Über β -Lävulin.)

28. ———, STEIGER, E., and MAXWELL, W. Zur Chemie der Pflanzenzellmembranen. *Zeitschr. physiol. Chem.* **14**: 227–273. 1890.
29. SELIWANOFF, T. Notiz über eine Fruchtzucker-reaction. *Ber. deutsch. chem. Ges.* **20**: 181–182. 1887.
30. SHERRARD, E. C., and BLANCO, G. W. Some of the products obtained in the hydrolysis of white spruce wood with dilute sulphuric acid under steam pressure. *Jour. Ind. Eng. Chem.* **15**: 611–616. 1923.
31. TOTTINGHAM, W. E., ROBERTS, R. H., and LEPKOVSKY, S. Hemicellulose of apple wood. *Jour. Biol. Chem.* **45**: 407–414. 1921.
32. ———, and GERHARDT, FISK. Quantitative methods for the analysis of hemicellulose in apple wood. *Jour. Ind. Eng. Chem.* **16**: 139–140. 1924.
33. VON FELLEBERG, T. Über den Nachweis und die Bestimmung des Methyl-alkohols, sein Vorkommen in den verschiedenen Nahrungsmitteln und das Verhalten der methylalkoholhaltigen Nahrungsmittel im Organismus. *Biochem. Zeitschr.* **85**: 45–117. 1918.
34. WINTERSTEIN, E. Über das Verhalten der Cellulose gegen verdünnte Säuren und verdünnte Alkalien. *Zeitschr. physiol. Chem.* **17**: 391–400. 1893.

INFLUENCE OF SHAPE ON THE CHEMICAL COMPOSITION OF POTATO TUBERS¹

J. J. WILLAMAN² AND ALICE M. CHILD

Decided differences in the shape of potato tubers of different varieties are a matter of common knowledge. It has been suggested that these variations in shape may affect the composition of the tubers. The evidence on this, however, is conflicting.

GLYNNE and JACKSON (3), investigating the distribution of dry matter and of nitrogen in potato tubers, dissected out the different zones and analyzed them. The main regions are the medulla, or inner, and the cortex, or outer region. They found that the percentage of dry matter is lowest in the skin, increases in the cortex, and then decreases in the medulla. The nitrogen content in these three is in the reverse order to that of the dry matter. COUDON and BUSSARD (1) obtained practically the same results.

EAST (2) states that the flatter tubers have a higher starch content, because of a greater proportion of cortex. Thus, if changing the shape changes the ratios of the zones, and if the latter differ in composition, it is fair to predict that the composition of the tuber as a whole will vary with the shape. On the above lines of reasoning WILLAMAN and WEST (8, 9) concluded that the more spherical tubers would have the higher protein content.

RENSKI (6), however, found a relation opposite to that above. He reports that the longer tubers have the lower starch content. His material included many varieties over a period of several years. More recently NEUMAN (5) also reports that the longer tubers have the lower starch content. He states that high nitrogen fertilization causes an elongation of the tubers, but he does not give any data on the nitrogen content of these tubers.

During 1922 and 1923 several lots of potatoes, including several varieties, were measured as to shape, and analyzed for dry matter and nitrogen. Although the data are somewhat scanty, they point rather definitely to the conclusion that the more spherical tubers have the higher protein content. Since it is improbable that the writers will have further data in the near future, it was thought best to publish the information so far obtained.³

¹ Published, by permission of the Director, as paper no. 868, Journal Series, Minnesota Agricultural Experiment Station.

² Now at Agricultural Experiment Station, Geneva, N. Y.

³ Acknowledgment is due to Sister M. BERNADETTE LIES, now at the College of St. Theresa, Winona, Minn., and to H. O. TRIEBOLD, now at State College, Pa., for the analytical data used in this paper.

Methods and materials

From 12 to 20 tubers were taken for a sample for analysis. The dimensions were taken in millimeters, and averaged for each lot of tubers. The tubers were scrubbed, dried, and weighed on a special balance in air and in water for determining specific gravity. Then they were shredded for analysis. Dry matter was determined on a small sample by drying at 100° C. Protein was determined on the dried and finely ground material by the Kjeldahl-Gunning method.

The five groups of samples involved were as follows, all of them grown in Minnesota:

A. Fourteen lots of Rural New Yorkers, grown in various parts of Minnesota in 1922.

B. Eight lots of Irish Cobblers grown in 1922 on peat, with various fertilizer treatments.

C. One lot each of King, Irish Cobbler, Green Mountain, Rural New Yorker, Burbank Russet, Bliss Triumph, Russet, and Early Ohio, grown on completely fertilized peat in 1922.

D. Eleven lots of Early Ohios, grown in various parts of Minnesota in 1922.

E. Twenty-six lots of Early Ohios, grown in various parts of the state in 1923.

Analysis of the results

Group E, of 26 samples, is the only one of any size for treatment statistically; since even this number is too small to justify the use of the ordinary coefficient of correlation, the coefficient of correlation by rank was calculated, using the following formula taken from JACKSON (4):

$$r = 1 - \frac{6 \sum D_k^2}{n(n^2 - 1)}$$

and the probable error by the formula:

$$e = 0.706 \frac{1 - r^2}{\sqrt{n}}$$

taken from WHIPPLE (7).

The results of these calculations for group E are given in table I. As has always been found by other workers, there is a high positive correlation between specific gravity and dry matter. Neither of these factors, however, appears to be related to nitrogen content or to any of the shape ratios in these samples. The nitrogen content of the fresh samples is negatively correlated with the length-depth ratio and with the width-depth ratio. In other words, the depth of the tuber is the deciding dimension; and as the depth increases, the percentage of nitrogen in the tuber as a whole increases.

TABLE I
COEFFICIENTS OF CORRELATION (BY RANK) AMONG SEVERAL FACTORS IN EARLY OHIO TUBERS (1923)
n = 26

	DRY MATTER	SPECIFIC GRAVITY	NITROGEN, FRESH BASIS	NITROGEN, DRY BASIS	LENGTH WIDTH	LENGTH DEPTH	WIDTH DEPTH
AVERAGE WEIGHT	-0.439 ± 0.112	-0.158 ± 0.135	-0.537 ± 0.098	-0.393 ± 0.117	+0.474 ± 0.107	+0.640 ± 0.082	+0.171 ± 0.134
DRY MATTER		+0.742 ± 0.062	+0.523 ± 0.100	-0.105 ± 0.137	-0.141 ± 0.136	-0.101 ± 0.137	-0.163 ± 0.134
SPECIFIC GRAVITY			+0.363 ± 0.120	-0.158 ± 0.136	-0.019 ± 0.138	-0.011 ± 0.138	+0.009 ± 0.139
NITROGEN FRESH BASIS					-0.182 ± 0.134	-0.333 ± 0.123	-0.345 ± 0.122
NITROGEN DRY BASIS					-0.111 ± 0.137	-0.167 ± 0.134	-0.282 ± 0.127
LENGTH WIDTH						+0.903 ± 0.025	-0.086 ± 0.137
LENGTH DEPTH							+0.421 ± 0.114

TABLE II

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "LONG" AND "SHORT" GROUPS ON BASIS OF LENGTH-WIDTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Long" class	5	4	6	11
"Short" class	9	4	5	15
Length-width ratio—				
Total range	1.28-1.07	1.15-1.04	1.47-1.21	1.43-1.27
Mean of "long" class	1.27	1.12	1.44	1.40
Mean of "short" class	1.15	1.07	1.26	1.33
Difference, per cent.	- 9	- 4	- 12	- 5
Width-depth ratio—				
Total range	1.34-1.20	1.34-1.25	1.14-1.10	1.15-1.09
Mean of "long" class	1.27	1.30	1.12	1.12
Mean of "short" class	1.24	1.29	1.12	1.13
Difference, per cent.	+ 2	0	0	+ 1
Length-depth ratio—				
Total range	1.64-1.36	1.54-1.34	1.66-1.35	1.61-1.42
Mean of "long" class	1.60	1.47	1.63	1.57
Mean of "short" class	1.43	1.38	1.42	1.50
Difference, per cent.	- 10	- 6	- 13	- 4
Dry matter—				
Total range, per cent.	25.7-20.0	22.8-17.9	25.9-22.3	24.4-20.7
Mean of "long" class, per cent.	23.4	20.2	24.3	21.9
Mean of "short" class, per cent.	22.5	21.7	23.8	22.6
Difference, per cent.	- 0	+ 7	- 2	+ 3
Nitrogen, wet basis—				
Total range, per cent.	0.543-0.350	0.518-0.326	0.529-0.410	0.499-0.377
Mean of "long" class, per cent.	0.400	0.416	0.456	0.422
Mean of "short" class, per cent.	0.457	0.436	0.479	0.434
Difference, per cent.	+ 14	+ 5	+ 5	+ 3
Nitrogen, dry basis—				
Total range, per cent.	2.50-1.61	2.27-1.53	2.21-1.72	2.17-1.73
Mean of "long" class, per cent.	1.77	1.99	1.88	1.92
Mean of "short" class, per cent.	1.98	2.00	2.03	1.94
Difference, per cent.	+ 11	0	+ 8	+ 1

This relation is not so evident when the nitrogen in the dry matter is taken for comparison.

Among the shape ratios, the length-width bears a very marked relation to the length-depth; and the latter bears a significant relation to the width-depth ratio. Thus the length is the dominating dimension. Furthermore, in comparing the weight of the tubers with the shape, it is evident that as the tubers increase in size it is largely because of increased length. This is to be expected from the fact that the newly formed tuber is almost spherical. It is surprising, however, that these data indicate less dry matter in the larger tubers. This is inconsistent with the insignificant coefficient of correlation between size and specific gravity. The larger tubers apparently have a lower nitrogen content, which is consistent with the low nitrogen and long tubers. A corollary of the above facts is that the highest nitrogen content may be expected in the smaller tubers and in those of more spheroidal shape. The nitrogen content calculated to the fresh basis gives more significant coefficients than the nitrogen on the dry basis.

(Groups A, B, and D, as well as group E again, were treated in a different way. The samples in each group were arranged in order of decreasing length-width ratio. Each group was then divided arbitrarily into two classes, one of high ratio and one of low. In each class the average value was calculated for each ratio, for the dry matter, and for the nitrogen content. These data are presented in table II. The class of high length-width ratio is termed for convenience the "long" class and the other the "short."

Although the number of samples in each class is small, comparisons will bring out general tendencies. Thus, the average width-depth ratios of the "long" and the "short" classes are not different, as indicated by the figures +2, 0, 0, and +1. The length-depth ratio seems to follow the length-width, however, as the differences between the two classes are -10, -6, -13, and -4 per cent. The dry matter is about the same for both "long" and "short" classes. The nitrogen on the fresh basis is consistently greater in the "short" classes. On the dry basis the tendency is less.

In table III the data are similarly arranged, but this time in respect to the width-depth ratio. Those of high ratio are called "flat," those of low ratio, "round" for convenience. Here it is seen that the nitrogen is consistently much higher in the "round" class.

In table IV the comparisons are made with respect to the length-depth ratio. Here again the "short" class contains much more nitrogen than the "long."

In all the above tables, group E exhibited the least range in shape ratios; and this explains the fact that it showed the lowest differences among the four groups in any one comparison.

TABLE III

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "FLAT" AND "ROUND" GROUPS ON BASIS OF WIDTH-DEPTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Flat" class	9	2	3	17
"Round" class	5	6	8	9
Width-depth ratio—				
Total range	1.34-1.20	1.34-1.25	1.14-1.10	1.15-1.09
Mean of "flat" class	1.27	1.34	1.14	1.14
Mean of "round" class	1.21	1.29	1.11	1.11
Difference, per cent.	- 5	- 4	- 3	- 2
Length-depth ratio—				
Total range	1.64-1.36	1.54-1.34	1.66-1.35	1.61-1.42
Mean of "flat" class	1.52	1.54	1.61	1.54
Mean of "round" class	1.44	1.39	1.50	1.52
Difference, per cent.	- 5	- 10	- 7	- 1
Dry matter—				
Total range	25.7-20.0	22.8-17.9	25.9-22.3	24.4-20.7
Mean of "flat" class	23.1	21.3	23.5	22.2
Mean of "round" class	23.9	20.9	24.2	22.6
Difference, per cent.	+ 4	- 2	+ 3	+ 2
Nitrogen, wet basis—				
Total range	0.543-0.350	0.518-0.326	0.529-0.410	0.499-0.377
Mean of "flat" class	0.417	0.370	0.445	0.420
Mean of "round" class	0.472	0.445	0.475	0.446
Difference, per cent.	+ 13	+ 20	+ 7	+ 6
Nitrogen, dry basis—				
Total range	2.50-1.61	2.27-1.53	2.21-1.72	2.17-1.73
Mean of "flat" class	1.86	1.74	1.89	1.89
Mean of "round" class	1.98	2.07	1.97	1.98
Difference, per cent.	+ 6	+ 20	+ 4	+ 5

The deductions from tables II, III, and IV are consistent throughout with those from the coefficients of correlation in table I. Thus three varieties of tubers show the same relations among the factors studied. For this reason it appeared justifiable to pool the data from all five groups, and calculate the ordinary coefficient of correlation between the nitrogen on the fresh basis and each of the shape ratios. The results are shown in table V. These coefficients on mixed varieties indicate the same relations as those on the Early Ohio alone: no significant value as regards length-width, but

TABLE IV

FOUR LOTS OF POTATO TUBERS SEGREGATED INTO "LONG" AND "SHORT" GROUPS ON BASIS OF LENGTH-DEPTH RATIO

CLASSIFICATION	RURAL NEW YORKER	IRISH COBBLER	EARLY OHIO	EARLY OHIO
	1922	1922	1922	1923
Group	A	B	D	E
No. of samples—				
Total	14	8	11	26
"Long" class	7	2	6	14
"Short" class	7	6	5	12
Length-depth ratio—				
Total range	1.64–1.36	1.54–1.34	1.66–1.35	1.61–1.42
Mean of "long" class	1.58	1.54	1.63	1.57
Mean of "short" class	1.41	1.39	1.41	1.49
Difference, per cent.	– 11	– 10	– 13	– 5
Dry matter—				
Total range	25.7–20.0	22.8–17.9	25.9–22.3	24.4–20.7
Mean of "long" class	23.6	21.3	24.3	2.21
Mean of "short" class	23.3	20.9	23.8	2.26
Difference, per cent.	– 1	– 2	– 2	+ 2
Nitrogen, wet basis—				
Total range	0.543–0.350	0.518–0.326	0.529–0.410	0.499–0.377
Mean of "long" class	0.405	0.370	0.439	0.423
Mean of "short" class	0.469	0.445	0.479	0.435
Difference, per cent.	+ 16	+ 20	+ 9	+ 3
Nitrogen, dry basis—				
Total range	2.50–1.61	2.27–1.53	2.21–1.72	2.17–1.73
Mean of "long" class	1.75	1.74	1.88	1.91
Mean of "short" class	2.06	2.08	2.03	1.93
Difference, per cent.	+ 18	+ 20	+ 8	+ 1

marked negative correlation as regards length-depth and width-depth. This emphasizes again that depth is the determining dimension so far as nitrogen content is concerned.

TABLE V

COEFFICIENTS OF CORRELATION BETWEEN NITROGEN CONTENT AND SHAPE RATIOS IN POTATO TUBERS

 $n = 67$

	$\frac{\text{LENGTH}}{\text{WIDTH}}$	$\frac{\text{LENGTH}}{\text{DEPTH}}$	$\frac{\text{WIDTH}}{\text{DEPTH}}$
Nitrogen (fresh basis)	$+ 0.0959 \pm 0.0816$	$- 0.2957 \pm 0.0752$	$- 0.4223 \pm 0.0677$

Conclusions

When the shape ratios of Early Ohio potato tubers are compared among themselves, it is found that there is a marked positive correlation between length-width and length-depth, and between length-depth and width-depth, but no relation between length-width and width-depth. Thus the length is the dominating dimension. It is also the dominating dimension when the shape ratios are compared with the size of tuber, indicating that as the tubers grow in size it is largely because of increased length. The nitrogen content of the tubers is governed more by the depth than by any other dimension. The smaller tubers tend to have a higher nitrogen content than the larger. These conclusions hold whether the comparisons are made within a variety, or among samples of tubers from several varieties.

If a variety of potato of high protein content be sought, it is believed that it will more likely be found in one having moderate sized tubers of spheroidal shape.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA.

LITERATURE CITED

1. COUDON, H., and BUSSARD, L. Recherches sur la pomme de terre alimentaire. *Ann. Sci. Agron.*, 2nd ser. **3**: 250-291. 1897.
2. EAST, E. M. A study of the factors influencing the improvement of the potato. *Illinois Agr. Exp. Station Bull.* 127: 375-456. 1908.
3. GLYNNE, MARY D., and JACKSON, VIOLET G. The distribution of dry matter and nitrogen in the potato tuber, variety, King Edward. *Jour. Agr. Sci.* **9**: 237-258. 1920.
4. JACKSON, DUNHAM. The algebra of correlation. *Amer. Math. Monthly* **31**: 110-121. 1924.
5. NEUMAN, H. Die Beeinflussung der Kartoffelknolle durch äussere Verhältnisse. *Jour. Landw.* **73**: 7-38. 1925.
6. RENSKI, M. D. Correlation between the longitudinal diameter of potato tubers and their starchiness. *Abs. in Exp. Sta. Rec.* **24**: 439. 1911.
7. WHIPPLE, G. M. Manual of mental and physical tests. 3rd ed. Baltimore. 1919. (P. 42.)
8. WILLAMAN, J. J., and WEST, R. M. Correlations among the constituents of potato tubers. *Proc. Soc. Exp. Biol. and Med.* **19**: 360-362. 1922.
9. ————. A statistical study of the composition of potato tubers. *Minnesota Studies in Plant Science* **5**: 211-227. 1925.

BRIEF PAPERS

THE WATER CONTENT OF WHEAT LEAVES AT FLOWERING TIME

ANNIE M. HURD-KARRER AND J. W. TAYLOR

(WITH ONE FIGURE)

When wheat plants are grown under certain constant growth conditions it has been reported¹ that the water content of their stems and leaves increases to a maximum at flowering time and decreases suddenly one or two days after the stamens have extruded. As an explanation the theory has been advanced that "the imbibition-capacity of the plasma colloids, which through the action of the growth-hormones is kept up as long as a vegetative growing point is present, decreases as soon as, through the act of fertilization, this growing point is virtually lost to the vegetative plant." As the suggestion is made that the results are of significance in connection with practical problems of irrigation during the flowering period, some determinations of the moisture content of normally developing field plants during this period may be of interest.

Dry-weight determinations were made at intervals of two or three days during the flowering and early maturation periods of plants of two fall-sown wheat varieties, Kanred and Purplestraw, which flowered and ripened on different dates. In the case of Purplestraw, there was a difference of six days in the time of flowering of two adjacent plots, the seed for which had been sown on different dates. This gave an opportunity to compare on the same days the moisture content of plants of one variety in different stages with reference to flowering.

Twenty primary tillers from each of the three plots, Kanred, Purplestraw no. 1, and Purplestraw no. 2, were cut close to the ground about every other morning, wrapped in paper to lessen evaporation, and taken to the laboratory, a short distance from the field on Arlington Experiment Farm, Rosslyn, Virginia. As rapidly as possible all the leaves were cut off at the ligule and those of each position were weighed separately.² Any external moisture present was removed by careful wiping with cheesecloth.

The material was dried at 100° C. for three days, by which time it reached practically constant weight. The procedure recommended by SANDE-BAKHUYZEN to eliminate sampling errors by dividing the values for the leaves by the leaf area was not followed. It has been found impossible

¹ SANDE-BAKHUYZEN, H. L. Studies upon wheat grown under constant conditions. *Plant Physiol.* 3: 1-30. 1928.

² The leaves were numbered from the base of the plant upward.

TABLE I

ABSOLUTE MOISTURE CONTENTS AND PERCENTAGES OF DRY MATTER OF LEAVES OF FIELD-GROWN WHEAT PLANTS DURING THE
FLOWERING AND MATURATION PERIODS^a

DATE (1928)	GREEN WEIGHT OF 20 LEAVES (IN GRAMS)				MOISTURE IN 20 LEAVES (IN GRAMS)				DRY WEIGHT (PERCENTAGES)			
	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES	1ST LEAVES	2ND LEAVES	3RD LEAVES	4TH LEAVES
May 22	Kandred (2.85) (1.65) (1.15) (1.04) (1.09) (0.91) (0.71)	6.26 (5.59) (4.60) (4.15) (3.64) (2.20) (1.72)	4.75 (4.75) (4.11) (3.96) (4.71) (3.48) (3.21)		(2.00) (0.87) (0.51) (0.40) (0.33) (0.22) (0.15)	4.79 (4.20) (3.47) (3.09) (2.39) (1.20) (0.93)	3.48 (3.39) (2.89) (2.82) (3.29) (2.41) (2.25)		(29.82) (47.27) (55.65) (61.54) (69.72) (75.82) (78.87)	23.48 (24.87) (24.57) (25.54) (34.34) (45.45) (45.93)	26.74 (26.63) (29.68) (28.79) (30.15) (30.75) (29.91)	
May 24		5.11 (4.91) (5.03) (4.56) (3.10) (3.02)	7.28 (6.96) (5.91) (6.43) (6.25) (5.85) (6.24)	5.26 (5.05) (4.30) (4.50) (4.47) (4.46) (4.69)	(3.45) (2.79) (2.48) (2.41) (2.27) (0.93) (0.85)	4.50 (3.94) (3.81) (3.89) (3.38) (2.13) (1.80)	5.63 (5.25) (4.47) (4.83) (4.59) (4.27) (4.98)	3.86 (3.55) (2.97) (3.14) (3.02) (2.95) (3.27)	(18.63) (21.85) (23.46) (22.40) (22.66) (25.88) (40.76) (42.57)	20.63 (22.90) (22.40) (22.66) (25.88) (31.29) (40.40)	22.66 (24.57) (24.37) (24.88) (26.56) (27.01) (20.19)	26.62 (29.70) (30.93) (30.22) (32.44) (33.86) (30.28)
May 26		4.86 (4.28) (4.21) (4.25) (3.07) (3.42)	3.96 (3.68) (3.82) (4.10) (3.15) (3.17)	2.33 (2.32) (2.28) (2.51) (2.12) (2.08)	(3.02) (2.37) (2.02) (2.07) (1.24) (0.84)	3.75 (3.13) (3.05) (3.07) (2.20) —	2.96 (2.65) (2.72) (2.92) (2.24) (2.21)	1.64 (1.57) (1.51) (1.67) (1.38) (1.35)	(22.96) (29.25) (31.06) (31.23) (37.37) (44.00)	22.84 (26.87) (27.55) (27.76) (28.34) —	25.25 (27.99) (28.80) (28.78) (28.89) (30.28)	29.62 (32.33) (33.77) (33.47) (34.91) (35.10)

^a An asterisk denotes date of flowering; parentheses, leaves partially yellow or dried; brackets, leaves completely withered.

to measure leaf area accurately during the maturation period after the leaves have begun to shrivel. Moreover, the leaves of each position from selected primary culms were fairly uniform in size, and individual variations in the rate of withering undoubtedly caused as much or more irregularity in the data than did variations in size. When as many as twenty leaves were taken for one sample, neither the small variations in total leaf area nor differences in degree of withering obscured the trends shown by the dry-weight measurements.

There is no evidence in these data of a maximum water content just before flowering nor of a sudden water loss after flowering. In general the water content of the leaves of both varieties was decreasing from the day the experiment was begun, which was nine days before flowering in the case of Kanred, and four days before in the case of Purplestraw no. 1. It is true that a relatively rapid decrease occurred just after flowering in the second and third leaves of Kanred, but a similar decrease occurred on the same dates in the lower leaves of Purplestraw no. 1, which was at that time seven days past flowering (fig. 1). Therefore the decrease in both varieties was apparently due to the warm, dry weather which prevailed at this time.³

The dry-weight percentages also give no evidence of a relation between the rate of drying and the date of flowering of these plants. In the case of Kanred, the lowest leaves were almost completely withered, and the second leaves partially withered, before flowering time. There was no significant change in the water content of the upper leaf, which remained green throughout the experiment. In the case of Purplestraw no. 1, there was no sudden increase in any of the leaves immediately after flowering. In Purplestraw no. 2, the percentages increased slightly for all leaves just after flowering, but the upward trend probably began before the first measurement, which was made just at flowering time. Furthermore, a similar increase occurred over the same period in the plants of the other two plots (excepting the completely withered first leaf of Kanred) and these plots were in pre-flowering stages.

SANDE-BAKHUYZEN states that in his experiments the first sign of the water loss after flowering was the yellowing of the tips of the leaves. Under field conditions yellowing of the leaves of wheat plants often begins before flowering, the time depending on environmental conditions and on the adaptability of the variety to the environment. In the present experiments the tips of at least the first leaves yellowed before flowering time in each of

³ The temperature and rainfall records were taken by Mr. A. MAYER at Arlington Farm, and are for 24-hour periods ending at 8:00 a. m. each day. Since the plants were always cut between 9 and 11 o'clock in the morning, the figures given in the graphs refer in each case to the 24-hour period preceding the date of sampling.

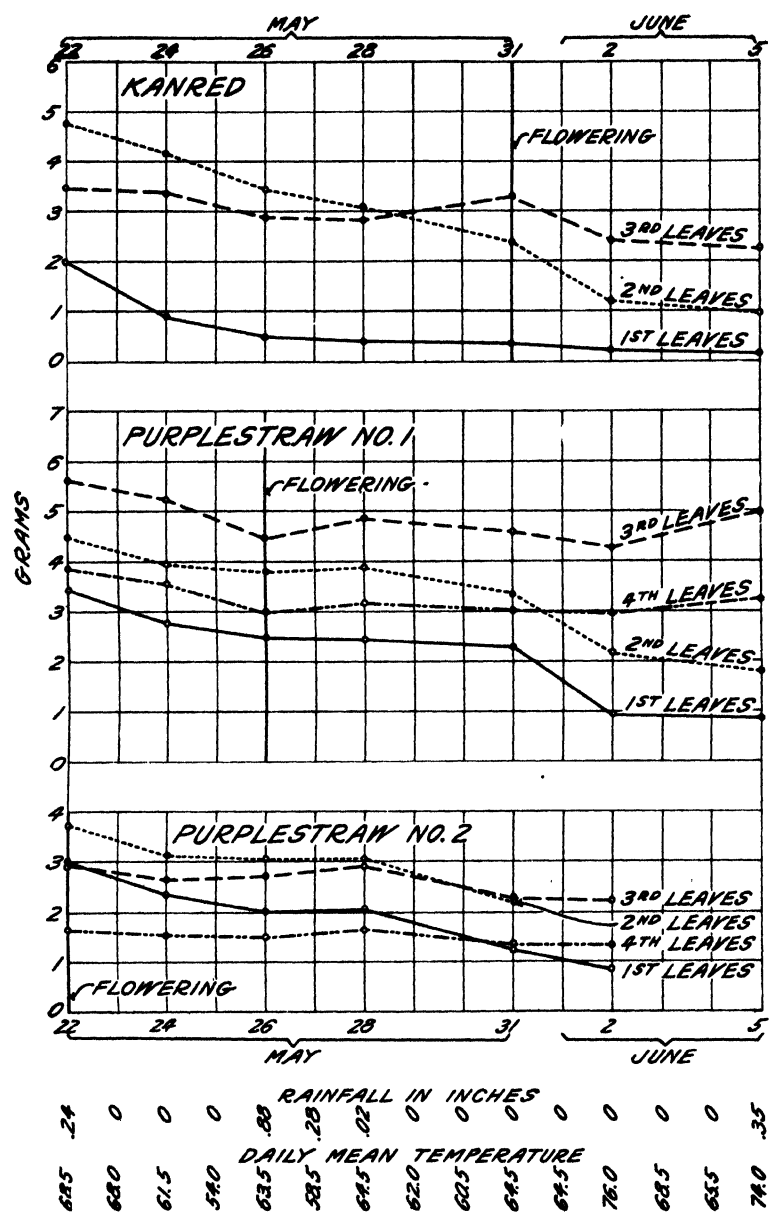


FIG. 1. Moisture in 20 leaves of each position on primary tillers of field-grown wheat plants during the maturation period.

the three plots. The lowest leaves of Kanred were completely withered seven days before flowering and the tips of the second leaves had begun to yellow six days before. In the case of Purplestraw, also, the first leaves were all beginning to turn yellow before the plants flowered.

Since the water content of the field plants fluctuated somewhat with changes in the external environment, some measurements were made on greenhouse plants grown under fairly uniform temperature and moisture conditions. It was possible to obtain tillers in pre-flowering, flowering, and post-flowering stages at one time and in the same rows. The data showed no differences in moisture content which were correlatable with differences in the stage of development of the tillers. Consequently, it appears that the physiological processes involved in flowering did not exert any specific effect on their water content.

Of course, the results of these experiments do not preclude the possibility that under a given set of conditions, such as those of a constant environment, desiccation might always begin immediately after flowering.

OFFICE OF CEREAL INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

NOTES

Annual Election.—The results of the annual election of the American Society of Plant Physiologists has been announced by the Secretary-Treasurer, Dr. H. R. KRAYBILL, as follows: For president, Dr. SCOTT V. EATON, of the University of Chicago; for vice-president, Prof. CHARLES A. SHULL, also of the University of Chicago. Because of previous service, the vice-president elect declined to serve, and the executive committee has elected Dr. A. E. MURNEEK, of the University of Missouri, to the vice-presidency. The new officers will have the hearty support of the entire membership. The growth of the Society during the last year has been very gratifying, and the financial condition at the close of the fiscal year on June 30 was stronger than at any time in the last four years.

Program Committee.—The program committee for the Des Moines meeting was appointed some time ago by the retiring president, Dr. E. J. KRAUS, of the University of Chicago. The committee is constituted as follows: Dr. W. E. LOOMIS, chairman, Iowa State College; Dr. J. P. BENNETT, Ohio Agricultural Experiment Station; Prof. L. F. GRABER, the University of Wisconsin; and Dr. H. R. KRAYBILL, of Purdue University, *ex officio*. All members of the Society are reminded of the desirability of concentrating our efforts on the Des Moines meeting. The program committee will appreciate the cooperation of all members, and each one should be prepared to respond promptly for any service that may be requested. With proper efforts the Society can greatly exceed any previous meeting in interest and enthusiasm.

Finance Committee.—The new constitution provides for a finance committee whose duties as stated are "to act in an advisory capacity to the Secretary-Treasurer with reference to the productive funds of the Society. The committee shall also seek ways and means of increasing the productive funds for support of the Society's work." The committee appointed by the President for this service includes Prof. CHARLES A. SHULL, chairman; Dr. WALTER THOMAS, Pennsylvania State College; and Dr. BURTON E. LIVINGSTON, Johns Hopkins University. The committee is appointed for three years, and the members will take their duties seriously. They will appreciate constructive suggestions at any time. An active program in furtherance of the purposes stated in the constitution will be initiated.

Life Membership Committee.—The selection of the fourth recipient of the CHARLES REID BARNES Life Membership in the American Society of Plant Physiologists has been entrusted by President SCOTT V. EATON to a

committee of members as follows: Dr. WALTER THOMAS, chairman; Dr. EARL S. JOHNSTON, University of Maryland; Dr. J. D. SAYRE, Ohio Agricultural Experiment Station; Dr. R. B. DUSTMAN, West Virginia University; and Prof. J. H. GOURLEY, Ohio Agricultural Experiment Station.

Portraits of Wilhelm Pfeffer.—As in the case of the portraits of SACHS and TIMIRIAZEFF, a number of portraits of PFEFFER have been printed without the plate number and name of the journal, to provide portraits suitable for framing. These copies are obtainable from the editor of PLANT PHYSIOLOGY at 12 cents each, postage prepaid. Stamps may be sent in payment.

Fall Meeting, American Chemical Society, Division of Colloid Chemistry.—The Division of Colloid Chemistry is to hold two half-day sessions at the fall meeting of the American Chemical Society in Minneapolis Sept. 9–13, 1929. The arrangements for the meeting are in the hands of Dr. F. E. BARTELL, of the Chemistry Department, University of Michigan, who is Chairman of the Division, and Dr. ROSS A. GORTNER, of the Division of Agricultural Biochemistry, University of Minnesota, Secretary-Treasurer of the Division of Colloid Chemistry. These meetings are valuable to anyone interested in the behavior of colloidal materials, and plant physiologists particularly would find them profitable.

International Horticultural Congress.—The ninth International Horticultural Congress will be held in London, England, from August 7 to August 15, 1930, just preceding the fifth International Botanical Congress, which is to be held at Cambridge, August 16–23, 1930. The Secretary of the Royal Horticultural Society, Mr. F. R. DURHAM, is secretary of the congress. The cost of membership in the congress will be one pound sterling, and should be sent to the Secretary of the Royal Horticultural Society, Vincent Square, London, S. W. 1. The main topic for discussion will be "Propagation, vegetative and seminal." An extensive program for visits to research stations and gardens of horticultural interest throughout the United Kingdom is being arranged. It will be a rare privilege for those planning to go to England in 1930 to attend both of these International Congresses.

Purdue Section.—It is a pleasure to record the progress of the Purdue Section of the Society. Their work has long since passed the experimental stage, and the value of sectional activity has been abundantly demonstrated. The last year was one of unusually interesting programs. The Section's meetings were held twice a month under the chairmanship of Dr. C. L. PORTER. The program was initiated with a dinner meeting at which Dr. ENDERS gave an interesting review of "Some recent advances in general physiology." The program was terminated with an address by Dr.

CHARLES F. HOTTES, Head of the Department of Botany, the University of Illinois. Dr. HOTTES chose as his subject for this address "The physiology of the cell." A group of fifty or more enjoyed his talk, dealing with recent research in relation to earlier work on the cell.

The year's program was divided in such manner that the first semester was devoted to a study of the nutrition of the higher plants, and the second semester to the nutrition of the fungi. The papers presented were as follows:

October 15, 1928, Some recent advances in general physiology. Dr. H. E.

ENDERS.

November 5, Nitrogen synthesis of higher plants. Dr. R. H. CARR.

November 19, Recent studies on carbohydrate synthesis. Dr. R. E. GIRTON.

December 3, Baron von Liebig's laboratory. Mr. H. F. HULSEMAN.

January 7, 1929, Joint evening meeting with the Biological Society. Some papers of interest at the New York meeting of the A. A. A. S.

January 14, Iron in plant nutrition. Mr. J. F. TROST.

February 4, Spore germination. Factors influencing germination, especially nutritive compounds, host influence, etc. Mr. COMPTON.

February 18, Relation of vegetative growth to nutrition of saprophytes. Mr. SAMPSON.

March 4, Establishment of nutritive connection in parasites (infection). Mr. DOAK.

March 18, Relation of parasites to the metabolism of their hosts. Effect of mineral nutrition, temperature, etc. Mr. WHITE.

April 1, Nature of resistance to parasites. Dr. E. B. MAINS.

April 15, Physiology of the cell. Dr. CHARLES F. HOTTES, University of Illinois.

Dr. R. H. CARR was elected chairman, and Dr. R. E. GIRTON secretary for the year 1929-1930.

Stephen Hales.—Admirers of STEPHEN HALES can now obtain an attractive biography of this interesting plant physiologist and natural philosopher. The biographer is Dr. A. E. CLARK-KENNEDY, Fellow of Corpus Christi, Assistant Director of the Medical Unit, and Assistant Physician to the London Hospital. The story of HALES's life and works is told in thirteen intensely interesting chapters, with fourteen illustrations. The frontispiece is a portrait of HALES, from the painting by Hudson in the National Gallery, London. Other illustrations show the Corpus Christi College which HALES attended, STUKELEY's map of the countryside around Cambridge and the Gogmagog Hills, where he and HALES botanized together, STUKELEY's drawing of HALES's orrery, a portrait of STUKELEY, a few plates of reproductions from *Vegetable Staticks*, Newgate prison,

equipped with a ventilator invented by HALES, and the parish church in Teddington where HALES served the spiritual needs of his people.

Every plant physiologist should know something of the historical background of plant physiology. HALES was the first great modern experimentalist in the field of plant physiology. The American Society of Plant Physiologists celebrated the 250th anniversary of his birth, and the 200th anniversary of his *Vegetable Staticks* at the Nashville Meeting in December, 1927, at which time a prize fund was established in honor of HALES.

The biography is an intimate account of a great scientist and a great humanitarian. The book is published by the Cambridge University Press, at \$6.00 per copy. Orders in this country should be sent to the Macmillan Co., New York.

Botany.—The science of Botany is being well served so far as the number of available new text-books is concerned. A recent addition to the literature of botany is the text by Dr. W. J. ROBBINS and HAROLD W. RICKETT, of the University of Missouri, entitled *Botany*, a text-book for college and university students. The approach to the subject is physiological. After a brief chapter on the cellular structure of plants, there are eleven chapters covering absorption of water and dissolved materials, the functions of roots and stems, leaf functions, food, energy relations, formation of new cells, growth, reactions to stimuli, life and death, and the origin of life.

The next twelve chapters consider the various groups of plants, beginning with the bacteria, yeasts, molds, and fungi. There are chapters on algae, ferns, bryophytes, club mosses, pine, and angiosperms (two chapters). The final chapters are on inheritance, evolution, and distribution. Review questions occupy 37 pages at the close of the text, and an appendix contains 101 titles, mainly of books, for reference. Only a few citations to periodic literature are included in the list.

The text is simply and clearly written. It seems to the reviewer a good beginning text for the general course in botany. Some of the topics are possibly treated in too elementary fashion for college and university students, particularly if they have had a year of botany in high school. It is better, however, to err on the side of simplicity than to be too technical in the first year college text. Teachers will find the book actually practicable in use, and students will find it interesting and stimulative. The price is \$3.75. Orders for the text should be sent to D. Van Nostrand Co., New York. A book of laboratory instructions by the same authors may be had to run along with the text.

The Physiological Basis of Drought Resistance.—Three years ago there was noted in these columns, under this title, the book which Prof. N.

A. MAXIMOV had just produced, and its forthcoming translation from Russian into English was announced. Owing to the illness of the editor, Prof. YAPP, this work was greatly delayed, and has only just appeared, with the title, *The Plant in Relation to Water*, and the subtitle as above. Prof. YAPP lived only long enough to see the last proof sheets, and never saw the book he edited from a sick-bed. The general plan of the book is unchanged from the Russian original, although the excellent account of sap ascent available in English permitted the elimination of chapters dealing with this, and those on cellular absorption have been condensed. Part I contains 75 pages, and treats in three chapters of The Absorption of Water by the Plant. Part II has 118 pages, and devotes four chapters to The Loss of Water from the Plant. Part III covers 184 pages and contains five chapters on The Water Balance and Drought Resistance of Plants. While the first two parts are admirably up-to-date and adequate in their treatment, the third part is the most significant portion of the book. And of this, the last two chapters are of especial interest, as embodying MAXIMOV's own results and theories regarding the relation between xeromorphism and drought resistance. Throughout the book, one is constantly impressed with the painstaking care of the author to include American and English work, as well as the more expected Russian and German work, and with the critical analysis given methods and results. This is no mere compilation, but a monograph by a recognized authority who has himself worked at the problem for fifteen years at first hand, and it deserves a wide reading by plant physiologists everywhere, both academic and applied. We owe a debt of gratitude to the author and the late editor for making available in concise form all the widely scattered data bearing on the water relations of plants, and especially the great amount of normally unavailable Russian work. It is a pity that so valuable a book is so poorly bound. There are 400 pages of text, a bibliography of 558 citations, and a good index. The publishers are Allen & Unwin, London, and the price from Macmillan Co., \$9.00. ♀♂

Fixation of Atmospheric Nitrogen.—A book of considerable interest to the biologist has been published on this subject by FRANK A. ERNST, of the Fixed Nitrogen Research Laboratory, U. S. Department of Agriculture. The book is concerned mainly with the commercial fixation of nitrogen, a chapter being devoted to each of the three main commercial processes, namely, the arc process, the cyanamide process, and the direct synthetic ammonia process. Equations showing the chemical reactions involved in these processes, and the energy relations of the reactions are given. Other chapters consider the various ammonia conversion products; economic considerations that are leading to the gradual replacement of Chile nitrate by the products of the commercial processes, and to the supremacy of the syn-

thetic ammonia process among the various commercial processes; statistics of the production of Chile nitrate and of the world production of inorganic nitrogen by the commercial processes. There are appendices giving the cost of production of Chile nitrate, the location of the world's plants for the fixation of atmospheric nitrogen, with annual capacity and 1927 rate of production, and nitrogen statistics of production, export, import and consumption for the principal countries of the world. The book contains 154 pages and is published by D. Van Nostrand Co., New York. The price is \$2.50.

PLANT PHYSIOLOGY

OCTOBER, 1929

COMPOSITION AND GROWTH INITIATION OF DORMANT BARTLETT PEAR SHOOTS AS INFLUENCED BY TEMPERATURE

F. E. GARDNER

(WITH NINE FIGURES)

Introduction

Considerable attention has been given in the past to methods of shortening the normal rest period of plants. The agents used to break the rest have included warm baths, nutrient salts, narcotics, anaesthetics, freezing and thawing, wounding, exposure to low temperatures, and other means of stimulation. However, in spite of the variety of means available for awakening the plant, we apparently have no convincing evidence as to how any of these agents bring about this renewal of growth. While it is not certain that all treatments which break the rest period act in a similar manner, it does not appear altogether unlikely that there may be a general principle involved: that a particular internal condition, within limits, must be attained before growth can take place.

Exposure to low temperature has long been recognized and employed as a successful means of terminating the rest period. The full significance of this natural means, however, is usually not appreciated, since in most temperate climates the season of dormancy is always accompanied by relatively low temperature, and the cold weather is commonly regarded as the cause for the lack of growth, whereas, it is probably the necessary antecedent to the growth of many plants.

In some regions, as in southern California, the winter months are not always accompanied by weather cold enough to bring about a normal spring awakening of all trees. This condition in the springs of 1904, 1924, and 1926, following unusually mild winters, prevailed to the extent that there were many acres of fruit trees completely dormant or growing indifferently

from only a few buds, when they should normally have been in full leaf. Crop failures resulted in the varieties which exhibited this trouble most markedly. HORNE, WELDON and BABCOCK (18), who have described this condition, are of the opinion that the trouble occurs every year in southern California to a limited extent and that it is not peculiar to California but is of general occurrence in very mild climates.

Various theories to account for the rest period and its termination have invoked changes in permeability, enzyme activity, food reserves, hydrogen ion concentration, and other possible factors. One of the most prominent has been the question of organic food supply, with its direct relation to enzyme activity. Since but little is known concerning the nature of the chemical changes accompanying the breaking of the rest, the present work was undertaken to ascertain the effect of temperature on some of the constituents which are ordinarily considered important in the metabolism of the plant and which may bear some relation to the termination of the rest period.

Review of literature

The literature pertaining to the rest period abounds in theories concerning the nature of the rest and in methods for its termination. These have been amply reviewed by HOWARD (19), WEBER (38), and APPLEMAN (2). The literature here referred to is concerned chiefly with the chemical changes accompanying the entrance into or the exit from the rest, and includes investigations on seeds, tubers, and woody tissues.

"After-ripening," which is characteristic of most seeds, and which is necessary in a greater or less degree for germination, is analogous to the rest period of woody plants. In some cases, delay in seed germination is due to the inhibiting nature of the seed coat, as CROCKER (7) has shown; but SHULL (36) has demonstrated that the delay is not necessarily entirely a seed coat effect, but may be due to a lack of stimulation of the embryo, as in the case of *Xanthium* seeds. DAVIS and ROSE (8), working with seeds of *Crataegus mollis*, have pointed out that an exposure to low temperature is an effective means of stimulating the embryo of this seed. Using the same temperature, 5-6° C., found by DAVIS and ROSE to be most suitable for the after-ripening of *Crataegus* seeds, ECKERSON (12) followed some of the metabolic changes occurring in the embryo of this seed during the after-ripening process. The food, she states, is stored in the form of fatty oils. There is also present considerable lecithin but no starch nor sugar. The initial change appears to be an increase in acidity, correlated with an increased water holding power and a heightened activity of catalase and peroxidase. She found that the after-ripening process could be greatly shortened by treating the embryos with dilute hydrochloric, butyric, or acetic acids.

PACK (29), in studying the after-ripening and germination of *Juniperus* seeds found that this seed, too, has a dormant embryo which must go through a series of changes before germination. For after-ripening, 5° C. seemed to be most favorable. The changes taking place at this temperature include an increase in hydrogen-ion concentration of the embryo, a dispersion of stored fats, a decrease in fats and proteins, an increase in sugar and amino acids, and a doubling of catalase activity. A later paper (30) appears to summarize PACK's rather inclusive view of the after-ripening process: "Accumulation of cell building materials: acids, phosphatides, active reducing substances, soluble sugars, pentoses, amino acids, soluble proteins, and other nitrogenous compounds; the accumulation of enzymes; the dispersion of materials; and the transformation of storage materials. This rapid accumulation of simple plastic cell materials, coupled with minimum respiration and combustion of materials, probably forces the dormant organs to activity. One thus sees the awakened active organ as a very unstable structure made up of many unstable compounds. If these changes are not the basis of the after-ripening process, they are found to accompany the after-ripening process."

A number of workers have demonstrated the importance of sugars for seed germination. IVES (20) tried various means of forcing seeds of *Ilex opaca*—a seed very difficult of germination; but no success was encountered with acids, bases, salts, electrical stimulation, etherization, light, oxygen, or enzymes. However, low temperature and treatment with a 5 per cent. dextrose solution were successful. Dextrose was reported to increase the germination from one in a million seeds up to sixty per cent. germination. KNUDSON (22) also has found sugars to be of assistance in the germination of orchid seeds. In this case fructose appeared to be more favorable than glucose.

The chemical changes found by various workers to occur in potato tubers are more consistent than those reported for seeds. MÜLLER-THURGAU (25) found an increase in the sugar content of tubers when they were subjected to low temperature. The tubers never formed sugar when frozen rapidly, but if their temperature was lowered slowly to -5° C. during fourteen days, the sugar content on the average increased from 0.3 per cent. to 0.92 per cent. At the same time more starch had disappeared than was necessary to account for the increase in sugar. He found no appreciable difference in the nitrogen determinations of chilled and unchilled potatoes. MÜLLER-THURGAU and SCHNEIDER-ORELLI (26) employed the warm bath method to hasten the germination of potato tubers and lily-of-the-valley bulbs. The warm bath, like low temperature, heightened the sugar content; but these workers do not attribute the hastened growth in this case to the

increased sugar, but, rather to some effect of high temperature on the protoplasm.

APPLEMAN (2) reports an increase in the reducing sugar content of potato tubers under the influence of low temperature. He compared two sets of tubers, one held constantly at a growing temperature, and the other at a variable low temperature. Analyses of these two sets were made from time to time throughout the storage period. No change was found in the amounts of diastase or invertase to explain the carbohydrate changes. He states that shortening of the rest in this case did not involve protein hydrolysis; for he found no appreciable differences between the two sets in the following nitrogen fractions—proteose nitrogen, peptones, amino acids, and amides. The various forms of phosphorus, too, remained constant until after germination had begun. APPLEMAN concludes that the rest period of potato tubers is not a firmly fixed and hereditary process, as many others (21) believe to be the case regarding this period in general.

BUTLER (4), HOPKINS (17), and DENNY (9) have also noted the increase in sugars in potato tubers following treatments which terminate the rest period. DENNY (9), in agreement with the findings of MÜLLER-THURGAU (25) and APPLEMAN (2), reports no consistent differences in various nitrogen constituents between awakened and dormant tubers.

Although low temperature, as an agent which breaks the rest period, has often been reported to increase the sugar content, ROSA (34) failed to obtain any shortening of the dormant period by low temperature. A high temperature (28–30° C.) did, however, have a marked accelerating effect on growth. LOOMIS and EVANS (24) suggest that in vegetative organs such as corms, tubers and woody twigs, which contain stored starch, the breaking of the rest period by ethylene chlorhydrin, ether and similar compounds is connected with the hydrolysis of the starch. Bulbs, on the other hand, which contain little or no starch, are not affected by treatment with these compounds.

Concerning the effect of temperature on the chemical changes taking place in woody tissues, less definite information is available than in the case of seeds and tubers. Perhaps the explanation is to be found in the relative ease of experimentation with these smaller organs. Although there are considerable chemical data indicating the seasonal changes in parts of trees throughout the year, it is not known what course these changes would follow under different temperature conditions.

PFEFFER, NEGER, DRUDE, JOST, JOHANNSSEN, SPÄTH, MAGNESS, and others (21) do not associate the rest period with chemical changes brought about by temperature effects, but believe that the yearly periodicity is the result of hereditary rhythm. KLEBS (21), on the other hand, considers it to be

the result of the relation of the plant to the outer world. The rest period begins, he believes, if, through a decrease in one or more factors—temperature, moisture, nutrient salts, etc.—the growth activity is lessened while assimilation continues until the accumulation of organic materials brings about an inactivation of the enzymes. HOWARD (19) who also favors this enzyme concept believes that all the phenomena connected with the entire resting phase are closely associated with the work of enzymes. He has demonstrated an increase in enzyme activity following various treatments which break the rest period. COVILLE (6), who also associates the breaking of the rest with the work of enzymes, has advanced a more specific view. He proposes that the starch stored in the tissues is separated from the enzymes by cell membranes; but, as the result of chilling, the vital activity of the membranes is weakened, and the enzyme “leaks through” to the starch and converts it into sugar. The high osmotic pressure resulting from the increased sugar initiates the growth of the buds.

While FISCHER (13) concludes that periodicity of growth is conditioned by a periodicity of the processes of food changes, he believes that these changes in turn rest upon an hereditary periodicity of certain properties of protoplasm. He followed the starch changes in the tree throughout the dormant season and found a starch maximum from leaf fall until November, then a decrease during the winter months, followed by a secondary maximum in April. PERETOLCIN (31) found that in the oak the period of greatest starch content corresponded to the period of deepest rest. Working with *Quercus*, *Fraxinus*, *Ulmus*, and *Tilia*, he reports a gradual conversion of starch into fats as the rest period draws to a close. WEBER (38) also reports an inverse relationship between starch and fat content in a number of species. TUTTLE (37), working chiefly with *Linnaea*, an evergreen species, claims that exposure to high temperature (20° C.) caused a conversion of oil into starch in the leaves, while a reconversion into oil was brought about by an exposure for eight days to 0° C. She states that in northwestern Canada, where chilling weather sets in early in the fall, the leaves of most evergreen trees are destarched by October and that they then contain large amounts of oils.

NIKLEWSKI (27), using excised branches of *Tilia*, *Prunus*, and *Betula*, quantitatively determined the amount of fats present in the branches before and after exposure to various temperatures from 1° to 22° C., and concluded that the fat content bears no relation to temperature. He does, however, report an increased sugar content in both bark and wood under the influence of low temperatures. POJARKOVA (32) found a relationship between the inversion of starch into sugar and the breaking of the rest, but reports complete absence of fats in the species investigated—*Berberis*, *Lonicera*,

Amelanchier, *Acer*, and *Corylus*. Evidently the conversion of starch to fats, sometimes reported to take place, is not of universal occurrence in all trees, nor does it appear to be indispensable to the breaking of the rest period.

KLEBS (21) believes that the rest period may begin because of lack of mineral nutrients to the buds. RIPPEL (33), however, calls attention to the fact that nitrogen and other elements are moved back from the leaves to the buds just before leaf fall, and that this increased mineral supply to the buds comes at a time when the rest period is beginning. While it is his opinion that the rest is something more than a matter of mineral supply, yet he shows the importance of mineral nutrients for continued development of the buds once their growth has been initiated.

ABBOTT (1) determined some of the chemical changes taking place at the beginning of the rest period in the tips of one-year-old peach seedlings and in the tips and bark of Grimes apple trees. The analyses show an increase during August, September, and October in sugars, starch, and total carbohydrates. The hydrogen-ion concentration of the expressed sap decreased in the fall and remained relatively low throughout the dormant season until growth began in the spring, at which time it again increased.

BORESCH (3) has shown that a high temperature coupled with insufficient oxygen leads to a considerable increase in free organic acids in plant tissue. This, he believes, is the effective action of the warm bath as a method of breaking the rest. The increased production of acids and the resultant change in pH of the protoplasm, may hasten bud development.

In regard to changes in sap concentrations, LEWIS and TUTTLE (23), working with sap expressed from evergreen leaves, found an increasing osmotic pressure during the winter and a subsequent falling off in the spring. The electrical conductivity during the same period remained fairly constant, indicating that the change in osmotic pressure was due to non-electrolytes. Sugar estimations on the sap followed in general the osmotic changes. GAIL (14) also reports that the density in the sap of leaves from pine and broad leaved evergreen shrubs increases during the winter months and reaches a maximum in late January and February.

This brief review of investigations concerning the changes associated with the breaking of the rest period serves to indicate the fragmentary and inconsistent nature of the evidence relative to this very important phase in the life of the plant.

Experimental material and methods

It is not meant in this section to include unnecessary details of procedures with which investigators are perfectly familiar. However, sufficient

details should be recorded to enable the reader to evaluate for himself the results obtained. This is particularly true of the analytical methods where a number of deviations from the usual procedures occur and where some justification of the methods used is necessary.

MATERIAL

Five-year-old Bartlett pear trees, grown in Berkeley, were used for this problem. These trees were heavily pruned in the early spring so that by fall they had produced an abundance of straight upright shoots, two and a half to three feet in length. These shoots constituted an unusually uniform group of material for analytical purposes.

On October 26, twenty-four trees were defoliated and dug from the orchard, as much of the root system being preserved intact as it was practicable to accommodate in the boxes of sand in which the trees were then planted. Eight of the trees, in boxes of moist sand, were placed in cold storage at a temperature of 2° C. A similar set of eight was maintained in the greenhouse, the temperature of which was controlled so that it never dropped below 16° C. The third set of eight was placed outside the greenhouse, thereby exposing the trees to the fluctuations of temperature characteristic of the winter season in Berkeley.

SAMPLING

Each sample collected for analysis consisted of twenty-four shoots, selected from the eight trees of the set with reference to uniformity of length, size, and position on the tree. The upper two feet of each shoot, constituting about three-fourths of its total length, were used for analysis.

The first three collections of shoots, dated September 11, October 2, and October 24, were taken from the selected trees before the trees were dug from the orchard in order to ascertain the normal trend of changes before subjection to different temperatures. After the trees had been dug and moved to the three temperature conditions previously stated, collections of shoots from the three sets of trees were made approximately every two weeks throughout the winter period in order to follow the chemical changes taking place in each set under its particular temperature condition.

In all cases bark was separated from wood for individual determinations. The samples were weighed and rapidly dried in a fan-ventilated oven at 78° C. and when dry, were re-weighed for moisture determinations and ground to a fine powder, most of the particles of which were able to pass a 100-mesh screen.

ALCOHOLIC EXTRACTION

5-gm. portions of the dry, powdered samples, were extracted in the Soxhlet apparatus with 95 per cent. alcohol to which had been added a small

amount of sodium carbonate. The extract was then entirely freed from alcohol by means of a hot air blast, taken up in warm water, filtered, and made up to a volume of 200 cc. with distilled water.

ANALYTICAL METHODS

Biological methods for the determination of the carbohydrates have been used as far as practicable. The use of enzymes instead of acids in the procedures involving hydrolysis has made "clearing" of the solutions unnecessary. Because of the methods employed it is believed that the fractions reported here represent rather closely the substances named—that the fraction termed "hexose sugar" represents only hexose sugar, and similarly for the other carbohydrate constituents.

Determination of reducing value.—The SHAFFER and HARTMANN (35) iodometric method for the determination of reducing value was employed with minor variations. In all cases the cuprous oxide precipitate was collected and washed on an asbestos mat in a Gooch crucible before it was dissolved and titrated. This process eliminated substances which may in themselves have no power of reducing Fehling's solution, but which might form addition or substitution products with iodine and thereby vitiate the titration. This precaution seems to be necessary in the case of pear tissues which contain large amounts of arbutin.

Total free reducing substances were determined on a 50 cc. aliquot of the uncleared solution.

Hexose sugar.—To a 50 cc. aliquot of the extract, a small cube of Fleischmann baker's yeast (approximately 0.3 gm.) and 4 cc. of 95 per cent. alcohol were added. The solution was shaken to get the yeast into suspension and then incubated 18–24 hours at 37° C. An occasional agitation during that period facilitated the fermentation of the hexose. It was found that yeast, by means of its emulsin, would split arbutin, which, as has been mentioned, is present in pear tissues, and particularly in the bark, in relatively large amounts. In preliminary work, this often resulted in a higher reducing value after fermentation than before, due to the hydrolytic product, quinol, not fermented by the yeast. The addition of alcohol checked the activity of the yeast emulsin, but did not interfere with complete hexose fermentation. The difference between the total free reducing substances and the reducing value after fermentation represents hexose sugar.

Non-hexose free reducing substances.—This fraction is represented by the reducing value obtained after all hexose material had been fermented away by the yeast.

Sucrose.—The increase in reducing value after invertase inversion is recorded as sucrose. Wallerstein's invertase scales were employed in this

determination, using 5 cc. of a 1 per cent. solution of the scales to 50 cc. of the plant extract.

It was found that "Difco" invertase was unsatisfactory when added to uncleared solutions, for a precipitation invariably resulted and often a decrease in reducing value instead of the expected increase. Presumably tannins or other substances capable of reducing Fehling's solution were precipitated by "Difco" invertase. The scales appear to be free from this objection. Incubation was carried out at 37° C. over night. In this case, also, 4 cc. of 95 per cent. alcohol was added to the solutions with the enzyme, since the invertase was not found to be free from emulsin which would otherwise split some of the arbutin present. The invertase scales have a small blank for which correction was necessary.

Arbutin.—For hydrolysis of this rather large fraction, the enzyme emulsin was used. Commercial emulsin seemed open to the same objection stated for "Difco" invertase, namely, that when added to uncleared solutions, precipitation resulted. A very active emulsin was prepared from sweet almonds, using the method described by ONSLOW (28). This preparation was not, however, free from invertase, and for this reason it was used in conjunction with invertase scales in order to be certain that complete inversion of the sucrose was effected as well as hydrolysis of the arbutin. Pure arbutin could be completely hydrolyzed by this emulsin within thirty-six hours at 37° C. The arbutin reported represents the difference between the reducing value after invertase inversion and that obtained after the use of emulsin plus invertase.

Starch.—To the sample residue, after extraction with alcohol, boiling water was added and the mixture allowed to boil for fifteen minutes on a hot plate. After cooling, 5 cc. of saliva and NaCl sufficient to make a 1 per cent. solution were added. Incubation was carried out at 40° C. for twenty-four hours. Continual agitation, by means of a shaker, facilitated the digestion.

Saliva was used for the digestion, in preference to Taka diastase, as being more specific for starch, since Taka diastase contains such a mixture of enzymes. Moreover, saliva has a zero blank with Fehling's solution, whereas, Taka diastase has a large one which becomes still larger if followed by acid hydrolysis.

Due to the presence of water-soluble acid-hydrolyzable material, not hydrolyzed by amylase and which therefore does not properly belong to the starch fraction, a factor, instead of the usual acid hydrolysis, was used to compute starch as glucose. The experimental curve (fig. 1), was constructed from data obtained by the use of potato starch and saliva under the same conditions of incubation given the plant samples. The reducing value in terms of cc. of thiosulphate after treatment with saliva is plotted

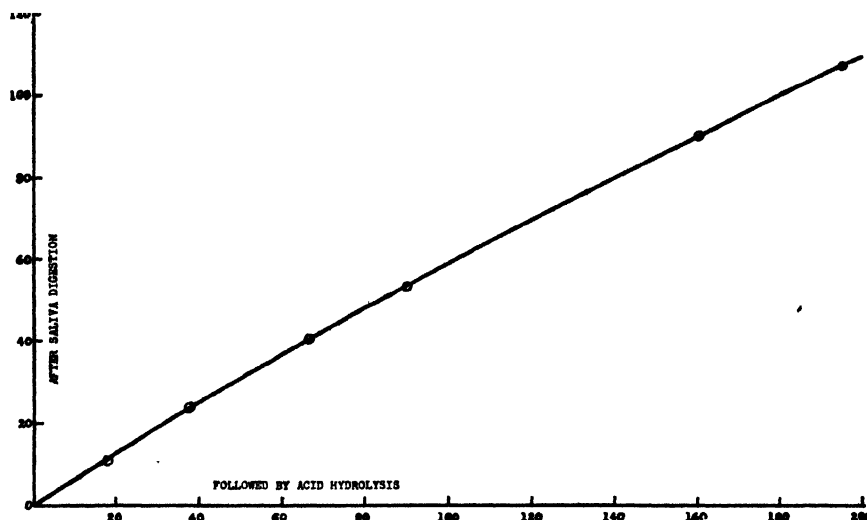


FIG. 1. Conversion curve for starch determinations. Reducing value in terms of cc. of thiosulphate after saliva digestion is plotted against value after acid hydrolysis of pure potato starch.

against the value obtained after acid hydrolysis of aliquots of the same samples. Using saliva in a constant amount, the factor, by which the reducing value after treatment with saliva must be multiplied in order to convert it into terms of glucose, is seen from the curve and from table I to increase as the amount of starch increases.

TABLE I

REDUCING VALUE OF INCREASING AMOUNTS OF POTATO STARCH AFTER SALIVA DIGESTION AND ACID HYDROLYSIS

AFTER SALIVA DIGESTION (THIOSULPHATE)	FOLLOWED BY ACID- HYDROLYSIS (THIOSULPHATE)	FACTOR
cc.	cc.	
11.6	17.8	1.534
23.9	37.7	1.575
40.5	66.0	1.63
53.6	89.0	1.66
90.5	160.0	1.77
107.0	195.0	1.82

Water-soluble polysaccharides other than starch.—After the determination of starch, a 50 cc. aliquot of the solution already digested by saliva was hydrolyzed with a 3 per cent. HCl for an hour in a boiling water bath

and then neutralized with NaOH. The difference between the reducing value after acid hydrolysis and the value from the starch conversion curve represents the water soluble polysaccharides.

Total nitrogen.—This determination was made by the Kjeldahl-Gunning method, using 5 gm. of dry sample.

Amide nitrogen.—This nitrogen fraction was determined by the standard method of SACHSSE, using 10 gm. of dry sample.

Freezing point depression.—These determinations were made with a Beckmann thermometer, on the sap immediately after its expression. The press used for obtaining the sap exerts a pressure on the sample of 870 kilograms per cm.², and the expression was continued until all the sap obtainable with this apparatus had been collected.

Electrical conductivity.—The conductivity of the expressed sap was determined by the standard method of Kohlrausch at 25° C.

Free organic acids and esters.—These were determined on 10 cc. portions of expressed sap which had been preserved in alcohol. An excess of 0.1 N NaOH was added and saponification carried out in a boiling water bath. The excess base was then titrated with standard acid, using phenolphthalein as an outside indicator. The natural color of the solution made detection of the end-point difficult; hence, the results are given as only approximate. Most of the acid found was combined, but it was not ascertained whether the acids of the fresh sap are largely combined, or whether esterification had taken place during the storage of the samples in alcohol.

Results

GROWTH RESPONSES

Cessation of the resting condition is not abrupt, but rather a gradual transition. The growth responses of orchard and storage trees reflected this gradual dissipation of the rest; and for this reason a sharp line demarcating the resting from the active condition can hardly be drawn through a series of frequent observations.

The experimental trees kept outdoors during the winter began growth in the spring at the normal awakening time. Digging of the trees in the fall evidently did not influence their time of exit from the rest period. Shoots were removed from these trees at frequent intervals throughout the winter and placed in jars of water in the warm greenhouse in order to follow the progress of the rest period. Shoots cut from the outdoor trees previous to December 7 exhibited practically no activity. Little chilling weather had been experienced up until this time. The collection of January 5 made a little growth after about three weeks in the greenhouse. This consisted chiefly of the unfolding of flower buds. Each successive collection

from these trees showed a quicker and more complete response than the last. By February 4 very fair response was secured within a week or ten days after transfer to the greenhouse, and it was judged that the rest period of the shoots from the orchard trees was over at this time.

Shoots from storage trees were capable of growth much earlier than those from the orchard set. By November 23, after four weeks in cold storage, a few flower buds opened within three weeks following the removal to the greenhouse. Here again each successive collection responded better and more quickly than the preceding one. By January 19, all buds showed considerable activity within four days after transfer to the greenhouse.

The absence of growth from the greenhouse trees was very striking. Although given warm temperature and abundance of moisture throughout the year these trees remained dormant, except for little tufts of growth around the stubs left after the removal of shoots. This growth, which was obviously the effect of wound stimulus, was decidedly localized. Except for a few terminal buds which now and then pushed out into a weak growth, there was no evidence of awakening on the part of the greenhouse trees after eleven months of external conditions favorable for growth. Shoots removed from these trees and placed in water also failed to awaken.

TEMPERATURE CONDITIONS IN BERKELEY

Figure 2 shows the temperature conditions in Berkeley to which the

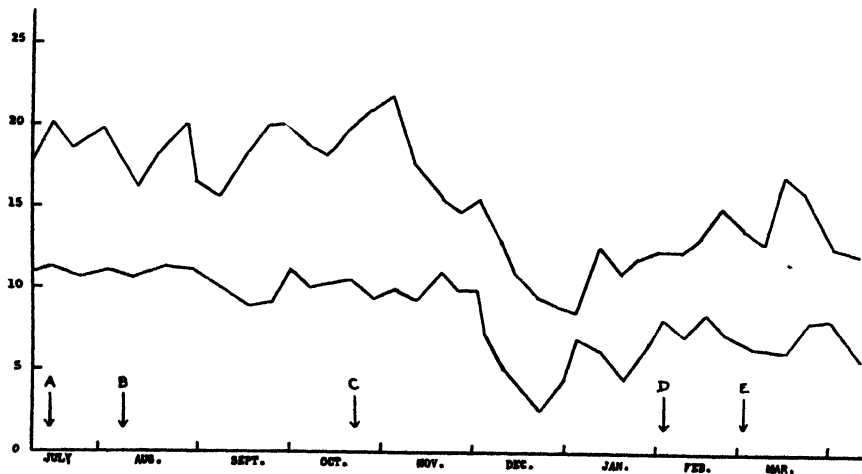


FIG. 2. Daily maximum and minimum temperatures in Berkeley, 1926-1927, averaged weekly. A, cessation of terminal growth. B, bark beginning to stick. C, trees dug. D, rest period over. E, buds swelling.

orchard trees were exposed. The state of activity of the trees is also indicated at intervals. Although the minimum temperature during the

winter months averages about 7° C., the lowest point in the curve being for the last week of December with an average minimum temperature of 2.5° C., yet this degree of chilling is apparently sufficient to bring about a normal spring awakening. Since the trees kept in the greenhouse, where the minimum temperature was 16° C., failed to awaken, it is apparent that the least chilling temperature which can break the rest period of the Bartlett pear tree lies roughly somewhere between 2.5° and 16° C. The length of exposure to a given temperature is probably important also. A longer exposure to only a moderately low temperature may be as effective in breaking the rest period as a shorter exposure to a lower temperature.

From the temperature curve it can be seen that the resting condition of the shoots was attained before the advent of chilling weather. This is in agreement with HOWARD's view (16), that the beginning of the rest is due wholly to inner causes.

ANALYTICAL RESULTS

Throughout the following tables and graphs the designations "orchard," "greenhouse," and "storage," respectively, refer to shoots taken from the trees maintained in the open with its uncontrolled temperature conditions, in the greenhouse where the temperature was never lower than 16° C., and in the storage at a constant temperature of 2° C. The first three collections of "orchard" material were made before the trees were dug; after which time, October 27, all trees were transplanted into boxes of sand and exposed to the temperature conditions stated for each set.

TABLE II
TOTAL MOISTURE, EXPRESSED IN PERCENTAGE OF FRESH WEIGHT

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
September 11 .	62.5			51.2		
October 2	59.9			45.3		
October 24	59.1			45.2		
November 4	59.0	59.1	60.3	45.8	47.8	46.5
November 23	59.4	58.6	59.8	46.0	44.9	46.8
December 7	58.0	58.0	58.3	45.6	44.8	46.9
December 23 .	58.3	63.0	58.5	47.0	46.2	47.4
January 5	58.6	59.4	58.2	46.8	46.9	47.1
January 19 .	58.6	58.7	58.4	47.8	47.0	47.6
February 4...	57.6	58.7	58.2	47.8	47.5	46.3
February 17 .	58.3	59.2	58.1	48.6	47.3	45.6
March 7 .	57.5	60.0	60.0	48.0	47.7	47.2

The moisture content throughout the period of experimentation is seen from table II to remain fairly uniform in all three sets of trees. Apparently a marked change in water content is not necessarily associated with the cessation of the rest period, nor with chemical transformations which take place under the influence of temperature. Because of the uniformity in moisture, expression of the following chemical data on a dry weight basis seems reliable for comparative results.

TABLE III
HEXOSE SUGAR, EXPRESSED AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11.	40.5			17.2		
October 2	41.4			24.5		
October 24	60.3			24.5		
November 4.	59.0	78.0	95.0	24.5	23.0	21.0
November 23.	53.0	72.0	108.0	24.5	19.0	39.0
December 7	70.0	60.0	99.0	27.0	21.0	36.0
December 23.	76.0	38.0	100.0	24.5	16.0	43.0
January 5.	87.0	50.0	118.0	26.0	21.0	50.0
January 19.	103.0	53.0	112.0	27.0	19.0	37.0
February 4.	123.0	55.0	123.0	23.0	16.0	35.0
February 17...	99.0	70.0	103.0	21.0	15.0	25.0
March 7	72.0	58.0	93.0	23.0	14.0	23.0

As regards the effect of temperature on the hexose content (table III and fig. 3) it is to be noted that the largest accumulation occurs under the condition of lowest temperature, while in the greenhouse trees hexose fails to increase.

The non-hexose free reducing substances indicate no constant differences between the three sets of trees. While it is not definitely known what constitutes this fraction, it may include among other substances, tannins, pentoses, and quinol. The variations in amount appear to be unaffected by temperature and to bear no relation to the changes accompanying the breaking of the rest period.

The effect of low temperature on the sucrose content is very similar to its effect on hexose sugar, although even more marked (table V and figure 4). Figure 5 expresses total sugars in each of the three sets of trees.

Arbutin, which assumes relatively large proportions in pear bark, appears to play no part, at least by variations in its amount, in the mechanism of growth-releasal. Under the influence of temperature it remains practi-

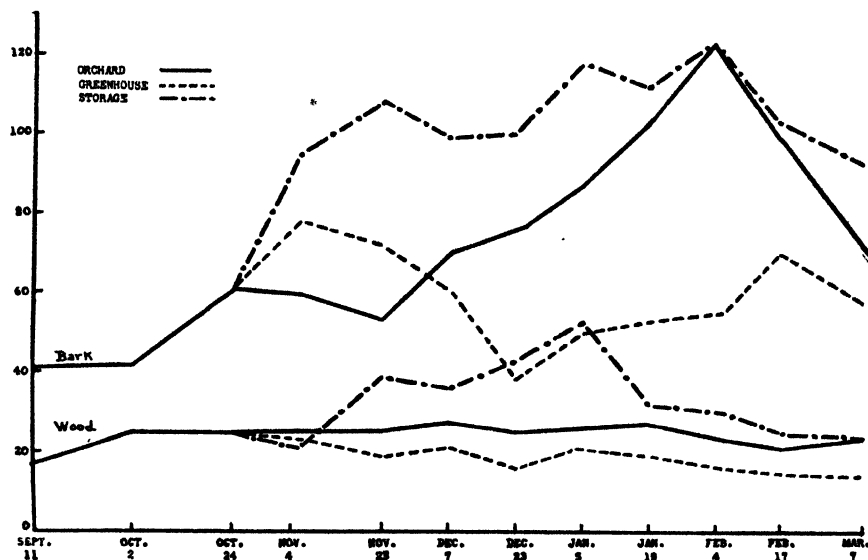


FIG. 3. Hexose sugar, as mg. of glucose per 5 gm. of dry sample.

cally unaffected (table VI). Some writers have postulated that glucosides are closely involved in the respiratory mechanism. If this is true it is interesting that arbutin remained so uniform under these widely different temperature conditions, which presumably resulted in marked differences in respiration.

TABLE IV

NON-HEXOSE FREE REDUCING SUBSTANCES, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	mg.	mg.	mg.	mg.	mg.
September 11	120			13		
October 2	120			11		
October 24	116			7		
November 4	126	110	127	19	8	11
November 23	126	95	121	12	8	15
December 7	117	106	114	13	11	12
December 23	148	110	130	9	13	11
January 5	144	112	123	16	9	11
January 19	133	118	127	21	9	11
February 4	128	136	127	23	11	12
February 17	124	123	145	15	11	11
March 7	142	134	145	19	11	15

TABLE V
SUCROSE, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11	5.0			6.0		
October 2	7.5			5.0		
October 24	9.0			7.0		
November 4	28.0	13.0	24.0	11.0	13.0	25.0
November 23	29.5	10.0	54.0	13.0	16.0	29.0
December 7	43.0	17.0	90.0	14.0	10.0	41.0
December 23	43.0	10.0	118.0	15.0	15.0	42.0
January 5	34.0	19.0	114.0	20.0	10.0	33.0
January 19	52.0	19.0	120.0	27.0	10.0	33.0
February 4	41.0	24.0	106.0	25.0	6.0	33.0
February 17	56.0	19.0	100.0	26.0	12.0	37.0
March 7	43.0	17.0	120.0	18.0	11.0	36.0

The changes in starch content at different temperatures are shown in table VII and figure 6. The starch increase in the fall, while the leaves were still on the trees, and the decrease from then on, characterize the curves. The greatest drop is seen in the storage trees—the same trees which showed the largest sugar increase. It is apparent, however, that the



FIG. 4. Sucrose, as mg. of glucose per 5 gm. of dry sample.

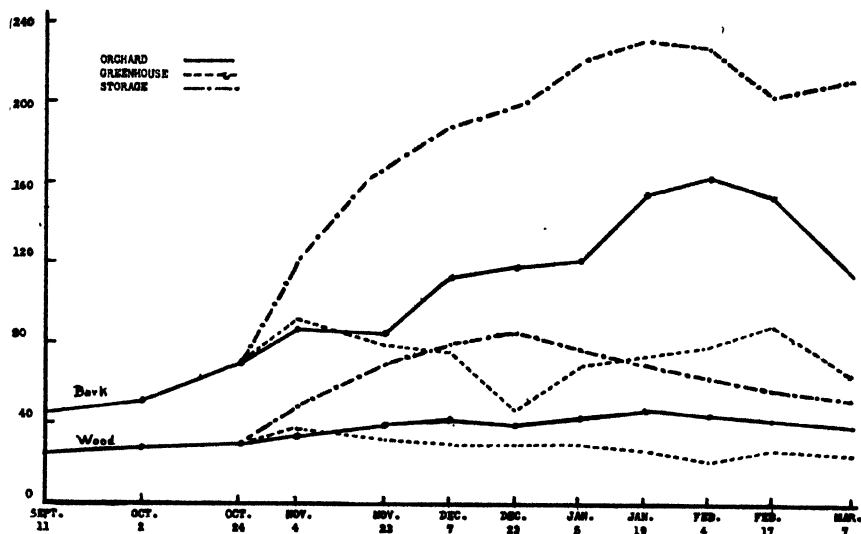


FIG. 5. Total sugars, as mg. of glucose per 5 gm. of dry sample.

decrease in starch is greater than is necessary to account for the increase in sugars. While this discrepancy might be accounted for entirely by respiration, there is still the possibility of starch conversion into some constituent not included in the analyses.

The fraction designated "water-soluble polysaccharides other than starch" is reported for pear bark only (table VIII). If present at all in

TABLE VI

ARBUTIN, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	mg.	mg.	mg.	mg.	mg.
September 11.	227			39		
October 2.	241			44		
October 24.	245			35		
November 4.	237	242	245	39	40	36
November 23	240	245	245	31	36	41
December 7	245	217	246	29	31	26
December 23	226	242	245	39	37	42
January 5.	226	237	222	40	42	38
January 19.	217	235	226	35	48	36
February 4.	214	192	222	41	42	40
February 17.	226	203	230	45	37	40
March 7.	237	202	222	39	36	37

TABLE VII
STARCH, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11 .	136			317		
October 2 .	185			505		
October 24 .	268			620		
November 4 .	224	210	163	684	705	615
November 23 .	185	200	108	625	676	560
December 7 .	166	200	90	625	615	520
December 23 .	163	200	80	596	573	495
January 5 .	154	186	80	570	548	476
January 19 .	145	198	83	517	515	455
February 4 .	133	175	80	470	476	437
February 17 .	111	175	71	455	480	446
March 7 .	111	169	71	461	476	420

the wood its amount is no greater than the experimental error of the starch determination. It is seen to be an erratic quantity, showing no particular trend and disclosing no relationship to the problem of the rest period. It

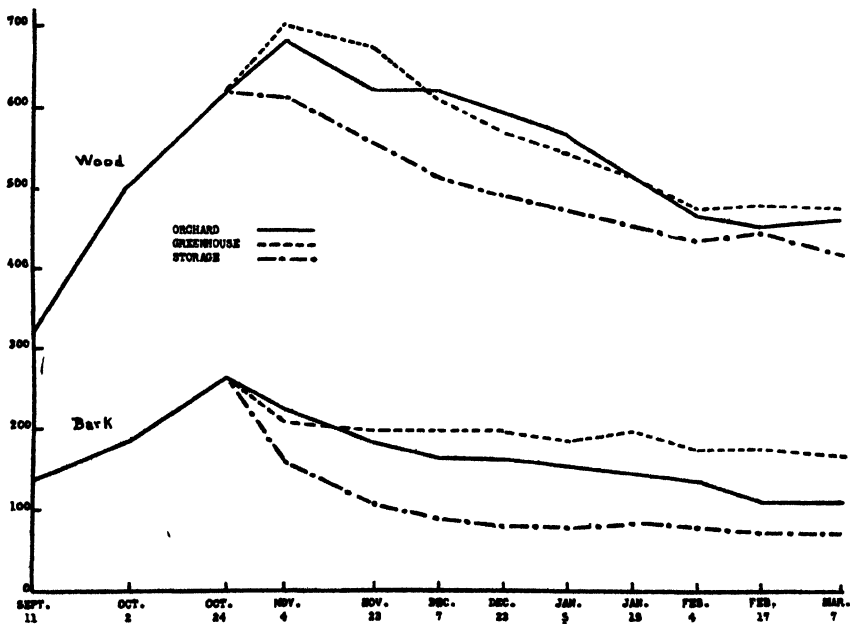


Fig. 6. Starch, as mg. of glucose per 5 gm. of dry sample.

TABLE VIII

WATER-SOLUBLE POLYSACCHARIDES OTHER THAN STARCH, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK		
	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11	72		
October 2	68		
October 24	68		
November 4	62	87	108
November 23	82	91	80
December 7	77	85	86
December 23	72	85	83
January 5	54	64	68
January 19	69	80	58
February 4	78	68	71
February 17	87	98	77
March 7	72	112	77

probably includes water-soluble hexosans and pentosans among other things. The table is included for the purpose of showing the magnitude of this fraction.

Complete data on the fat content of the shoots was not obtained. However, benzene extractions were made on material collected January 19 from three sets of trees and showed no appreciable difference between the three sets. At the time of this collection, other constituents were showing maximum differences as the result of temperature. In all cases the weight of the benzene extract was very small and appeared to be largely due to pigments.

Total nitrogen (table IX), as might be expected of trees standing in pure sand, remained practically constant. There is perhaps a tendency on the part of the bark of orchard and greenhouse trees to increase in nitrogen, but it will be observed that the wood of these trees decreases in nitrogen about the same extent. Possibly there was a migration of nitrogen from wood to bark at these temperatures, whereas at the low storage temperature no such transfer took place. In any event, temperature apparently does not influence the total nitrogen of the entire shoot, nor is the total amount of nitrogen in itself a factor related to growth renewal.

Little can be concluded regarding the relation of the amide nitrogen fraction to the growth responses of the trees from the three sets (table X). There does, however, appear to be some indication from the data of orchard

TABLE IX
TOTAL NITROGEN, AS MG. PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11 .	50.5			30.5		
October 2 .	51.5			28.6		
October 24 .	53.3			28.2		
November 4 .	54.2	52.5	53.3	28.0	29.4	29.4
November 23 .	53.8	54.8	52.9	26.3	28.4	29.0
December 7 .	53.3	55.5	52.0	25.3	28.0	29.8
December 23 .	54.8	55.7	54.3	22.7	26.0	28.4
January 5 .	56.5	57.8	52.9	22.4	25.8	28.0
January 19 .	58.1	58.5	53.0	22.2	25.2	28.1
February 4 .	58.0	60.1	54.0	23.1	25.0	28.4
February 17 .	60.4	59.5	53.7	23.5	25.2	28.3
March 7 .	61.2	58.7	54.3	24.4	24.1	29.4

trees that amide nitrogen is relatively high in the fall while the trees are active, is lower during the dormant season, and again increases as growth begins in the spring. The vernal increase, however, did not take place until *after* growth initiation.

The changes in freezing point depression of the expressed sap under the three temperature conditions are shown in table XI and figure 7. The

TABLE X
AMIDE NITROGEN, AS MG. PER 10 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
September 11 .	6.30			4.55		
October 2 .	5.03			3.14		
October 24 .	4.75			2.50		
November 4 .	4.55	5.23	4.98	2.16	2.32	2.94
November 23 .	4.75	5.20	4.31	2.27	2.10	2.82
December 7 .	4.53	4.55	4.33	2.13	2.21	2.97
December 23 .	4.98	5.03	4.86	1.76	2.12	2.66
January 5 .	5.06	4.98	4.62	1.76	2.10	2.66
January 19 .	5.06	5.06	4.84	1.99	2.10	2.50
February 4 .	4.90	5.06	4.28	1.85	2.10	2.80
February 17 .	5.03	4.90	4.15	1.88	1.88	2.72
March 7 .	5.25	4.65	4.55	1.96	2.13	2.82

TABLE XI
FREEZING POINT DEPRESSION OF EXPRESSED SAP

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>
September 11 .	1.11			0.58		
October 2	1.25			0.56		
October 24..	1.22			0.49		
November 4.	1.20	1.22	1.25	0.51	0.54	0.56
November 23 .	1.21	1.28	1.39	0.55	0.54	0.62
December 7 .	1.26	1.25	1.58	0.53	0.52	0.70
December 23	1.31	1.28	1.65	0.55	0.62	0.75
January 5	1.43	1.29	1.65	0.58	0.61	0.76
January 19	1.43	1.28	1.59	0.62	0.55	0.75
February 4	1.46	1.24	1.64	0.64	0.57	0.76
February 17 .	1.39	1.21	1.63	0.59	0.54	0.75
March 7	1.33	1.24	1.71	0.58	0.55	0.80

differences in freezing point depression probably can not be attributed to electrolytes, since the electrical conductivity remains uniform. It is interesting that the freezing point curves follow in general the curves for

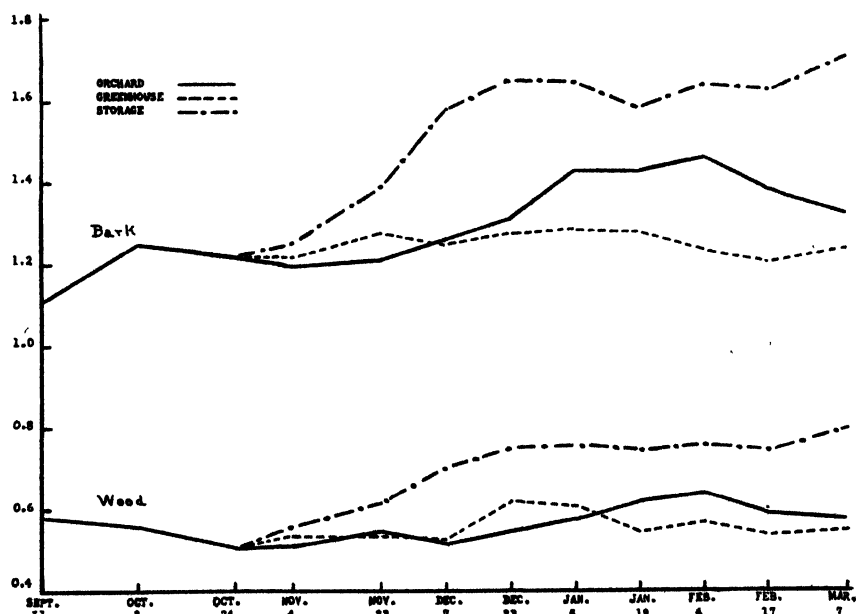


FIG. 7. Freezing point depression of expressed sap, in degrees centigrade.

hexose and sucrose. Although the sugars are the only sap-soluble constituents found thus far to undergo changes comparable to the changes in freezing point depression, it does not appear likely that they could be wholly responsible for such marked freezing point differences. For this reason the depressions which would be contributed by the sugars have been subtracted from the experimental freezing point determinations, and the curves thus redrawn to exclude sugar effects (fig. 8).

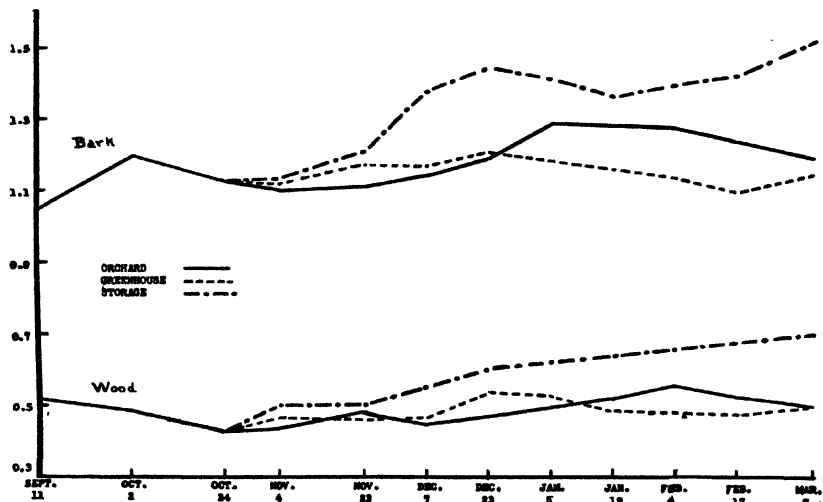


FIG. 8. Freezing point depression of expressed sap minus sugar effects.

The revised freezing point curves make it apparent that some sap-soluble substance or substances not yet accounted for show marked changes under the influence of low temperature, and that they simulate the behavior of the sugars. This observation led to the investigation of organic acids, or their esters, in the expressed sap (table XII).

The organic acids of the sap, or their esters, occur in rather large proportions, equivalent in some instances to a 0.4 N solution. Low temperatures appear to increase the amount of this fraction, the effect being similar to the behavior of the sugars though not so marked (figure 9). It is believed that the remaining difference found in the freezing point depression curves after the deduction of sugars can largely be accounted for by this fraction, although as explained before, this determination of acids is only an approximate one. The results are, however, sufficiently reliable for the conclusion that low temperatures result in appreciable changes in the acid content of the sap, these changes being similar to those which take place in the sugars and in the freezing point depression.

TABLE XII

ORGANIC ACIDS OR THEIR ESTERS, EXPRESSED AS CC. OF 0.1 N ACID PER 10 CC. OF SAP

DATE	BARK		
	ORCHARD	GREENHOUSE	STORAGE
	cc.	cc.	cc.
November 4	28.3	28.0	30.8
November 23	28.7	29.0	35.0
December 7	29.3	28.5	36.2
December 23	30.3	29.0	38.0
January 5	31.6	29.0	37.7
January 19	30.2	28.4	37.0
February 4	34.2	28.0	39.1
February 17	33.8	27.0	39.0
March 7	33.5	28.4	41.2

Discussion

The Bartlett pear tree, after subjection to sufficient chilling, shows a very definite growth response which does not occur if the temperature remains relatively high during the dormant season. Trees which were moved into the warm greenhouse in October, without previous chilling, were still practically dormant eleven months later, although temperature and moisture conditions were favorable for growth at all times. Exposure to low temperature apparently brings about a change in some internal condition which is necessary for the beginning of growth.

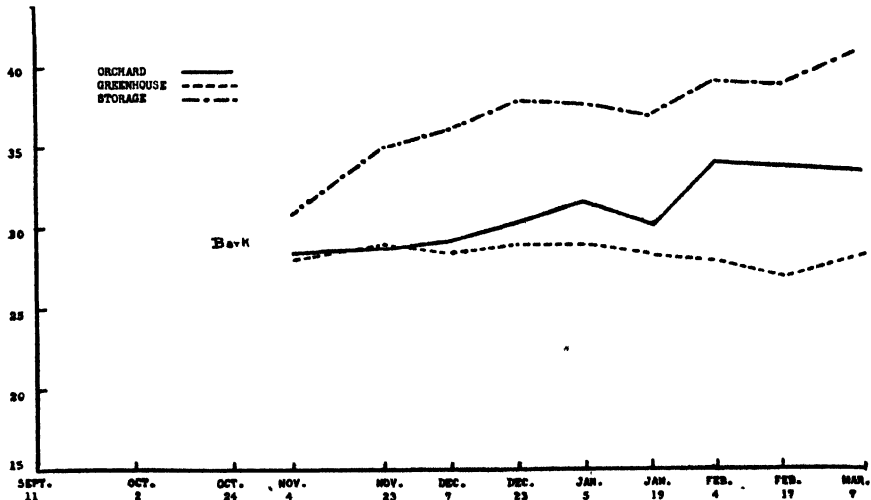


FIG. 9. Organic acids, or their esters, as cc. of 0.1 N NaOH to neutralize 10 cc. of expressed sap.

The changes in composition which are induced by exposure to relatively low temperatures are found to occur chiefly in the carbohydrates. The increase in sugars, although very marked and correlated with the ability of the shoots to grow, has not been established as necessary for the growth response. COVILLE (6) believes that a high concentration of sugar initiates the growth of the buds by means of the high osmotic pressure thus produced. APPLEMAN (2), on the other hand, considers the increase in sugars, resulting from exposure to low temperature, to be no essential part of the after-ripening process in potato tubers. Although a supply of soluble carbohydrates is necessary for growth, there is no positive evidence that a high accumulation of sugars must occur before growth can begin. However, the greenhouse trees did not accumulate sugars and did not grow.

The starch in all trees was shown to decrease after the fall maximum, but not to the same extent under each temperature condition. The greatest decrease occurred at the lowest temperature. Presumably the starch was converted into sugar, and the accumulation of sugars at low temperature is perhaps the result of a retarded rate of respiration. Although enough starch disappeared from the greenhouse trees to bring about a high concentration of sugar, respiration at the greenhouse temperature may have been so rapid that sugar could not accumulate. In the pear shoot the difference in sugar content between the three sets of material may thus be largely due to a difference in the respiratory rate, although it has been shown that the disappearance of starch is more marked at the lower temperatures.

The secondary starch maximum, reported by FISCHER (13), HOOKER (16), DU SABLON (11), and others, to occur in the early spring, was not found in the pear shoots under any of the temperature conditions. CAMERON (5), using microchemical methods, reports this secondary starch maximum in the Bartlett pear to take place in February just before the beginning of growth. A secondary starch maximum in leafless trees would presumably be accompanied by a decrease in sugars or other carbohydrates. Usually the amount of sugar present in the plant is not enough, if all converted into starch, to result in the marked starch regeneration often reported. Whatever the source of the secondary starch maximum, it appears unrelated to the cessation of the rest period, for both storage and outdoor trees exhibited the ability to grow without first showing a second increase in starch.

It was previously mentioned that the increase in fat content, reported by a number of investigators to take place during the winter months, does not appear to be of universal occurrence in all trees subjected to low temperatures. Apparently it does not occur in shoots of the Bartlett pear tree. NIKLEWSKI (27) found no supporting evidence of such a change in branches of *Prunus*, *Betula*, or *Tilia* under different temperature condi-

tions; and POJARKOVA (32) has reported an absence of fats in a number of deciduous species. It appears that a change in fat content does not always accompany the ending of the rest period.

In so far as the nitrogenous constituents were examined, the results agree with the findings of APPLEMAN (2), MÜLLER-THURGAU (25), and DENNY (9), that the cessation of the rest period is apparently not dependent upon nitrogen transformations. It is not felt, however, that the incomplete partition of total nitrogen here effected is adequate for a definite conclusion regarding this point. Although no appreciable changes were found in the total and amide nitrogen under the different temperature conditions, the amide fraction as determined constitutes such a small part of the total nitrogen that important changes may have occurred in the other forms of nitrogen which make up the large remainder.

All the constituents of the pear shoot here investigated, with the exception of starch, are much more abundant in the bark than in the wood. That this is true, also, of total soluble organic materials and electrolytes is shown by the freezing point depression and electrical conductivity of expressed sap. Not only is the bark richer in food substances, but the greatest changes under the influence of low temperature occur here. Perhaps this is explainable in that bark has a relatively greater proportion of living cells than wood. It is possible that a finer division of tissues, than the gross separation of bark from wood, should have been made before analysis. HOWARD (19) believes that the secret of the rest period resides in the buds themselves rather than in the cambium or any other tissues of the branch. HODGSON (15) found that the breaking of the rest by etherization was accompanied by a disappearance of starch from the buds.

By means of chemical stimulation, DENNY and STANTON (10) have awakened individual buds on a shoot without influencing the remaining buds. They state that the rest period is not systemic but is localized in the buds and that, therefore, any analyses to elucidate the rest period should not include other tissues. The fact should be recalled, however, that individual buds can also be awakened by injury to the stem. Injury to the bark, such as ringing, notching or bruising, will initiate the growth of buds immediately below the injury although these buds, themselves, are not touched. The writer inclines to the view that the rest period is systemic in the sense that it is common to the meristems of the entire plant whether in the bud, stem or root, and that any localized portion of the meristem will renew activity when the conditions either within that portion of meristem or in the tissues surrounding it are favorable for growth.

The theory that enzyme inactivity in the fall is responsible for the beginning of the rest period and is the result of high organic accumulation, and that enzyme reactivation, when that accumulation has been respired

or otherwise removed, initiates growth in the spring, has found favor with a number of writers. In this work, however, there seems to be no evidence that enzymes could be inactivated in the fall by a high organic accumulation, or that reactivation in the spring is resumed because of the removal of that accumulation; for in both the fall and the spring there was found the reverse of the conditions proposed by the theory as regards accumulation of soluble organic materials. In the fall, throughout the period when the pear shoots were presumably entering the rest period, hexose, sucrose, and freezing point depression, which is indicative of the amount of soluble material, were relatively low; while at the time of the cessation of the rest period, the accumulation of soluble organic materials was at a peak. The accumulation of insoluble substances, such as starch, would be less liable to retard enzyme activity.

Since the changes in composition reported in this work were presumably brought about by enzymes, it is interesting, in view of the large temperature coefficients for enzyme action, that the most pronounced changes were found in the trees held constantly at such a low temperature, while the least change occurred in the trees maintained at a high temperature. Although differences in the rate of respiration have probably modified the changes occurring, certain enzyme activity was apparently greater at the low temperatures; for example, the disappearance of starch and also the formation of sucrose were greatest at 2° C. Perhaps, within the plant, the chemical equilibrium points of various reactions which are catalysed by enzymes shift with changes in temperature, or possibly the amounts of enzymes produced at the different temperatures, rather than the activity of a constant amount, may be responsible for the effect. APPLEMAN (2), however, reports no difference in the amounts of diastase or invertase activity in chilled and unchilled potato tubers, yet marked changes in the carbohydrates occurred. COVILLE's concept (6), that the enzymes are separated from their substrata by membranes which become more permeable after sufficient chilling, appears to explain the results, although it is without supporting evidence.

It is apparent that further work is necessary in order to explain the effect of low temperature in bringing about the growth of plants. Several other avenues of attack on this problem of the rest period suggest themselves as being of promise. While this work indicates the changes which occur, or fail to occur, in a number of plant constituents, it does not preclude the possibility of important changes in constituents other than those investigated. In addition to a more complete analysis than was made in this work, the changes which occur in the various tissues might be determined. As has been pointed out, it is not known in what tissue or tissues the secret of the rest resides and a gross analysis of bark and wood may

serve to dwarf changes in some constituents which may actually be significant in a particular tissue.

Perhaps some indication as to whether sugars, organic acids, or other substances which increase under low temperature, are essential to the breaking of the rest, may be gained by introducing them singly or in combination into dormant trees. The after-ripening of seeds has apparently been shortened by treatment with sugars (20) (22) and acids (12).

In addition to exposure to low temperatures, other methods which break the rest period—warm baths, etherization, etc.—could be studied in a similar manner to determine their effect on the constituents of the plant. Perhaps certain changes in composition would be found common to all methods of stimulation. From the convergent lines of evidence then available a satisfactory conclusion might be reached regarding what internal conditions of the plant are essential for the renewal of growth.

Summary

1. Dormant Bartlett pear trees were maintained throughout the winter and spring months at different temperatures. Some of the trees were kept in cold storage at a constant temperature of 2° C.; others in the greenhouse where the temperature was never lower than 16° C.; still others at the variable intermediate temperature conditions outdoors.

2. Trees kept in the warm greenhouse remained practically dormant throughout the year, while those exposed to cold storage and to outdoor conditions became capable of growth. The storage trees, which were subjected to a lower temperature than outdoor trees, were first to finish their rest period.

3. At frequent intervals shoots were removed from the trees for analysis in order to ascertain the changes in certain constituents taking place under each of the temperature conditions.

4. The low temperature of the storage and the relatively low outside temperature, both of which resulted in a breaking of the rest period, brought about the following changes in the composition of the shoots: an increase in hexose sugar, sucrose, organic acids, and freezing point depression of the expressed sap, while starch markedly decreased. Little change in these constituents occurred in the trees held in the warm greenhouse.

5. No effect of the different temperature conditions was found in the following determinations: non-hexose reducing substances, water-soluble polysaccharides other than starch, arbutin, fats, total nitrogen, amide nitrogen, and electrical conductivity of expressed sap.

6. The changes reported as the result of exposure to low temperature have not been established as necessary to the initiation of growth; however, growth did not take place in the absence of these changes.

The author gratefully acknowledges the helpful advice of Dr. J. P. BENNETT of the University of California, in whose laboratory this work was done.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF MARYLAND.

LITERATURE CITED

1. ABBOTT, O. Chemical changes at beginning and ending of rest period in apple and peach. *Bot. Gaz.* **76**: 167-184. 1923.
2. APPLEMAN, C. O. Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. *Bot. Gaz.* **61**: 265-294. 1916.
3. BORESCH, K. Zur Analyse der fröhrtreibenden Wirkung des Warmbades. *Biochem. Zeitschr.* **153**: 313-334. 1924.
4. BUTLER, O. A note on the significance of sugar in the tubers of *Solanum tuberosum*. *Bull. Torrey Bot. Club* **40**: 110-118. 1923.
5. CAMERON, S. H. Storage of starch in the pear and apricot. *Amer. Soc. Hort. Sci.* **20**: 98-100. 1923.
6. COVILLE, F. V. The influence of cold in stimulating the growth of plants. *Jour. Agr. Res.* **20**: 151-160. 1920.
7. CROCKER, WM. Rôle of seed coats in delayed germination. *Bot. Gaz.* **42**: 265-291. 1906.
8. DAVIS, W. E., and ROSE, R. C. The effect of external conditions upon the after-ripening of the seeds of *Crataegus mollis*. *Bot. Gaz.* **54**: 49-62. 1912.
9. DENNY, F. E. Chemical changes induced in potato tubers by treatments that break the rest period. *Amer. Jour. Bot.* **16**: 326-337. 1929.
10. ———, and STANTON, E. N. Localization of response of woody tissues to chemical treatments that break the rest period. *Amer. Jour. Bot.* **15**: 337-344. 1928.
11. DU SABLON, LECLERC. Recherches physiologiques sur les matières de réserves des arbres. *Rev. Gen. Bot.* **16**: 339-368, 386-401. 1904.
12. ECKERSON, S. A physiological and chemical study of after-ripening. *Bot. Gaz.* **55**: 286-299. 1913.
13. FISCHER, A. Beiträge zur Physiologie der Holzgewächse. *Jahrb. wiss. Bot.* **22**: 73-160. 1891.
14. GAIL, F. W. Osmotic pressure of cell sap and its possible relation to winter killing and leaf fall. *Bot. Gaz.* **81**: 434-445. 1926.
15. HODGSON, F. R. Observations on the rest period of deciduous fruit trees in a mild climate. *Proc. Amer. Soc. Hort. Sci.* **20**: 151-155. 1923.

16. HOOKER, JR., H. D. Seasonal changes in the chemical composition of apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 40. 1920.
17. HOPKINS, E. F. Relation of low temperatures to respiration and carbohydrate changes in potato tubers. Bot. Gaz. 78: 311-325. 1924.
18. HORNE, W. J., WELDON, G. P., and BABCOCK, E. B. Resistance of peach hybrids to an obscure disease in southern California. Jour. Hered. 17: 98-104. 1926.
19. HOWARD, W. L. An experimental study of the rest period in plants: Physiological changes accompanying breaking of the rest period. Missouri Res. Bull. 21. 1-72. 1915.
20. IVES, S. A. Maturation and germination of seeds of *Ilex opaca*. Bot. Gaz. 76: 60-77. 1923.
21. KLEBS, G. Über das Treiben der einheimischen Bäume, speziell der Buche. Heidelberg. Akad. Wiss., math. naturw. Kl., Abh. 3: 1-114. 1914.
22. KNUDSON, L. Non-symbiotic germination of orchid seeds. Bot. Gaz. 73: 1-25. 1922.
23. LEWIS, F. J., and TUTTLE, G. Osmotic properties of some plant cells at low temperatures. Ann. Bot. 34: 405-416. 1920.
24. LOOMIS, W. E., and EVANS, M. M. Experiments in breaking the rest period of corms and bulbs. Proc. Amer. Soc. Hort. Sci. 25: 73-79. 1928.
25. MÜLLER-THURGAU, H. Ueber Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Landw. Jahrb. 11: 751-828. 1882.
26. ———, and SCHNEIDER-ORELLI, O. Beiträge zur Kenntnis der Lebensvorgänge in ruhenden Pflanzenteilen II. Flora. 104: 386-446. 1912.
27. NIKLEWSKI, B. Untersuchungen über die Umwandlung einiger stickstoffreicher Reservestoffe während der Winterperiode der Bäume. Beihefte Bot. Centralbl. 19 Abt. 1; 68-117. 1905.
28. ONSLOW, M. W. Plant biochemistry. Second ed. p. 162. 1923.
29. PACK, D. A. After-ripening and germination of *Juniperus* seeds. Bot. Gaz. 71: 32-60. 1921.
30. ———. Chemistry of after-ripening, germination, and seedling development of *Juniper* seeds. Bot. Gaz. 72: 139-150. 1921.
31. PERETOLCIN, K. Die Änderungen von Reservestoffen bei unseren Bäumen in der Winterruheperiode. Mitteil. des Petersburg. Forst.-Inst. 11: 3-42. 1904.
32. POJARKOVA, A. Winterruhe, Reservestoffe und Kälteresistenz bei Holzpflanzen. Ber. deutsch. bot. Ges. 42: 420-429. 1924.

33. RIPPPEL, A. Untersuchungen über die Mobilisation der Aschenbestandteile und des Stickstoffs in Zweigen beim frühjährlichen Austreiben. *Biochem. Zeitschr.* **113**: 125-144. 1921.
34. ROSA, J. T. Relation of tuber maturity and of storage factors to potato dormancy. *Hilgardia* **3**: 99-124. 1928.
35. SHAFFER, P. A., and HARTMANN, A. F. The iodometric determination of copper and its use in sugar analysis. *Jour. Biol. Chem.* **45**: 349-390. 1920.
36. SHULL, C. A. The oxygen minimum and the germination of *Xanthium* seeds. *Bot. Gaz.* **52**: 453-477. 1911.
37. TUTTLE, G. M. Induced changes in reserve materials in evergreen herbaceous leaves. *Ann. Bot.* **33**: 201-210. 1919.
38. WEBER, F. Untersuchungen über die Wandlungen des Stärke- und Fettegehaltes der Pflanzen, insbesondere der Bäume. *Sitzungsber. Kais. Akad. Wiss. Wien.* **118**: 967-1032. 1909.
39. ————. Studien über die Ruheperiode der Holzgewächse. *Sitzungsber. Kais. Akad. Wiss. Wien.* **127**: 57-91. 1928.

RELATION BETWEEN THE DEVELOPMENT OF ROOT SYSTEM AND SHOOT UNDER LONG- AND SHORT-DAY ILLUMINATION

J. E. WEAVER AND W. J. HIMMEL

(WITH SEVEN FIGURES)

Relatively few investigations have been made to determine the factors which affect the relation of the growth of roots to tops. A more exact knowledge of the relations between aerial and subterranean plant parts and the degree to which these may be modified through cultural practices is of great scientific and practical importance. Extensive investigations have shown that plants exhibit marked specific and varietal differences with respect to relative development of roots when grown under the same environmental conditions. Intensive studies on the growth of wheat by WEAVER, KRAMER, and REED (20) and of certain other plants by CRIST and STOUT (3) have also made clear that there is a persistent tendency towards a positive correlation between roots and shoots, increase in size of tops being accompanied by increase in size of roots. Although significant variations in the relative distribution of the growth rate of tops and roots occur in nature and may readily be induced by change in environment, the fact remains that there is a persistent positive correlation in size of tops and roots regardless of the wide variations induced by special conditions.

The maintenance of a proper balance between root and shoot is of very great importance. If either is too limited or too great in extent, the other will not thrive. The root system must be sufficiently widespread to absorb enough water and nutrients for the stem and leaves, which, in turn, must manufacture sufficient food for the maintenance of the root system. The plant is a biological unit although it is frequently not treated as such. It is often mutilated by pruning, cutting or injuring the root system, frequently without much regard to the effect upon the remaining portion.

JEAN and WEAVER (7), HARRIS (6), and others have shown that the general effect of decreased water content of soil, providing enough is available to promote moderate growth, is to accelerate root development in relation to growth of tops. Low humidity resulting in high transpiration gives similar effects. MAXIMOW and LEBEDINCEV (13), MAXIMOW *et al* (14), and TURNER (18), have found that plants grown in shade have a less extensive root system and a greater ratio of dry weight of tops to roots. MOELLER (15), GERICKE (5), and LIVINGSTON (9) have shown that in sterile soils or in dilute nutrient solutions, roots develop extensively in relation to

tops and the ratio of dry weight of tops to roots is greatly decreased. Conversely, a generally enriched soil or one with an abundance of nitrates increases the ratio of tops to roots (6, 18, 3). CHANDLER (2), for example, found that "while the nitrate has increased both top and root growth, it has not increased root growth to as large an extent as it has increased top growth. Thus, while the top growth of fertilized trees [peaches] is twice as great as that of the unfertilized trees, the root growth is only 50 per cent. greater." As pointed out by TURNER (18), the decreased root growth in the presence of an abundance of nitrogen may result from a low supply of carbohydrates. As regards the roots of seedlings, it has been shown that their development is greatly influenced by the nature of the reserve food supply in the seed. The more nitrogen a seed contains, the greater is its shoot growth as compared to roots. REID (17) has shown that an abundance of carbohydrate foods and a somewhat limited nitrogen supply promote rapid root development.

Cultural practices such as pruning the tops, etc., may affect the normal correlation between the development of root and stem in a very definite manner. AUSTIN (1), for example, reports experiments with 3-year-old almond trees where the development of both the tops and root systems was inversely proportional to the severity of the pruning of tops. The spread of the roots was over one-third greater where the pruning was light than where it was severe.

The preceding data are sufficient to indicate that a plant is a very plastic organism and that any factor that influences the development of either root or shoot may also profoundly affect the other. The extensive researches of GARNER and ALLARD, which have since been supplemented by those of several other investigators, show clearly the profound effect that the period of daily illumination exerts upon the vegetative and reproductive activities of plants. However, so far as the writers are aware, relatively little attention has been given to the effect upon the root systems. GARNER and ALLARD (4) state that "preliminary observations indicate that the duration of the daily illumination period may exert a marked effect on the relative development of the root and the aerial portions of the plant. For example, a cutting of Biloxi soybeans made no top growth at all through the winter months and the original leaves assumed a very dark color and generally unthrifty appearance. Apparently new buds were unable to develop. Upon examination of the underground portion of the plant in the spring it was seen that the soil contained a large mass of roots altogether out of proportion to the top of the plant, as judged by the usual summer growth. Other similar cases have been observed in which a light duration unfavorable to aerial development has caused extensive root growth.

Growth of root and shoot, therefore, are not necessarily contemporaneous with respect to season, and arrested development of the exposed portion of the plant caused by suboptimal light duration need not be accompanied by checking of root growth."

Under periods of illumination varying from 5 to 24 hours duration, PFEIFFER (16) found that the amount of development in the fibrous root systems of tomato and buckwheat appeared roughly comparable with that of the aerial parts of the same plants.

JOHANSSON (8) showed that root development, in proportion to the total weight of the plant, increased with an increase in light intensity. For intensities of 39 and 70 per cent. there is a falling off in the rate of increase, in proportion to total weight, at 10 or more hours daily illumination and for an intensity of 23 per cent. a decrease begins at 12 hours daily illumination.

LUBIMENKO and SZEGLOVA (12) found that the weight of the roots increased in proportion to the weight of the whole plant as the length of the daylight periods became progressively longer.

CRIST and STOUT (3) grew plants in 6-inch pots at East Lansing, Michigan. They found in the case of lettuce and radish that shortened periods of illumination (6 hours daily) gave a greater ratio of tops to roots, based on dry weight, than either the normal length of day in cloudy weather of winter or one prolonged 6 hours by means of artificial illumination. Moreover, the plants grown under the longest period of illumination had the lowest top-root ratios while the short-day plants had the lowest actual weight of both tops and roots.

Undoubtedly the most significant criterion for judging the efficiency of root development is measurement of the actual absorbing surface. Because of the great difficulties of procedure this has been accomplished only in a few instances (20, 19). The method of determining dry weights has a twofold handicap. Where roots are grown in the soil it is practically impossible to free them completely from adhering rock particles without losing some of the roots. But a much more serious objection is encountered in the fact that this method when applied to maturing plants gives little idea of root efficiency. The woody tissues of a single main root that had ceased functioning often far outweighs an extensive network of actively absorbing rootlets. Consequently, it was deemed best in these experiments to use containers sufficiently large to hold a mass of soil in which the roots could develop in a somewhat natural manner. By accurately measuring and comparing root lengths and diameters, and the number, size, and degree of branching, a close approximation to the relative root development of plants grown under different conditions was obtained. To check out variations within the individual, several plants of each species were grown.

Procedure

In these experiments, the following plants were employed: red clover (*Trifolium pratense*), white icicle radish (*Raphanus sativus*), an iris (*Iris germanica*), common sunflower (*Helianthus annuus*), yellow duke dahlia (*Dahlia pinnata*), great ragweed (*Ambrosia trifida*), white Kherson oats (*Avena sativa*), and an early flowering cosmos (*Cosmos bipinnatus*). These were grown in heavy galvanized containers, 30 cm. square and 60 cm. deep, into the top of each of which was inserted a tight wooden frame in such a manner that the soil depth was increased 20 cm. The crevices were luted with plasticine. The containers were filled with a rich loam soil of optimum and uniform water content, which was well screened and exceptionally free from partially decayed roots or other debris. The water-holding capacity of the soil was about 50 per cent. (Hilgard method) and the hygroscopic coefficient 8.2. In filling the containers, the soil was moderately compacted, and the original water content was maintained by adding water from time to time in sufficient amounts to restore the original weight. Two containers were used for each species.

The ragweeds were transplanted from the field in the early seedling stage without injury to the root system. Iris was propagated by rhizomes 1 to 1.5 inches long, carefully selected for uniformity in size, the leafy tops being cut back to within 4 inches of the rhizome. The dahlias were grown from uniformly selected fleshy roots, and all of the other species from seed. After sowing or transplanting, the soil was covered with a fine gravel mulch to retard surface evaporation. The dahlias were planted on May 3, cosmos and clover on May 5, iris on May 8, and the others on May 16. On May 20, one container of each species was placed on an improvised truck so arranged that they could be placed alongside of the other containers from 9:00 A. M. until 4:00 P. M. each day and kept in an adjacent, well ventilated dark room the rest of the time. Thus, one-half of the plants were subjected to a short day of 7 hours' duration, and the rest to the normal 13- to 15-hour day during May and June.

Results

RED CLOVER

The 20 clover seedlings in each container had developed only a single leaf per plant when the difference in length of day was imposed upon them. Within a week the long-day plants had twice the stature of those of the short-day and thereafter differentiation was marked. On June 19, when the containers were opened and the roots excavated, the long-day plants were 28.4 cm. high and in full bloom but the paler colored short-day plants were only 9.8 cm. tall (fig. 1). Leaflets of the short-day plants averaged 1.75 by 2 cm.; those of the long-day 2.5 by 3.5 cm. The larger plants had



FIG. 1. Long-day and short-day plants of red clover 7-weeks old. The long-day plants are in blossom.

25 leaves each, the smaller ones only 12. The result of this difference in photosynthetic surface was clearly shown in the dry weight of tops which was 34.1 and 4.1 grams for the two lots respectively.

Red clover develops a strong taproot from which major laterals arise mostly in the surface 15 cm. of soil. These extend outward more or less horizontally or obliquely and then turn downward and penetrate deeply. At greater depths the primary laterals are smaller and do not spread widely.

The taproots on the short-day plants were only 1.5 mm. thick as compared with 4 mm. for those on the long-day plants. All but one, which barely reached the bottom of the container, ended at 62 cm. or above, while all those of the larger plants extended to and ran along the bottom of the container. The number of major laterals on the proximal 15 cm. of taproot was 3 to 5 per plant, and the total number of laterals was about 40 in both cases. On the short-day plants, however, very few of these extended below the 25 cm. level, a maximum depth of 45 cm. being determined. But those of the other group were as large as the taproots of the smaller plants and most of them extended much deeper, often running along the bottom of the container. The long-day plants alone had root branches of the third order. Below 15 cm. depth, laterals on the short-day taproots were mostly 1 cm. or less in length while both the main roots and primary

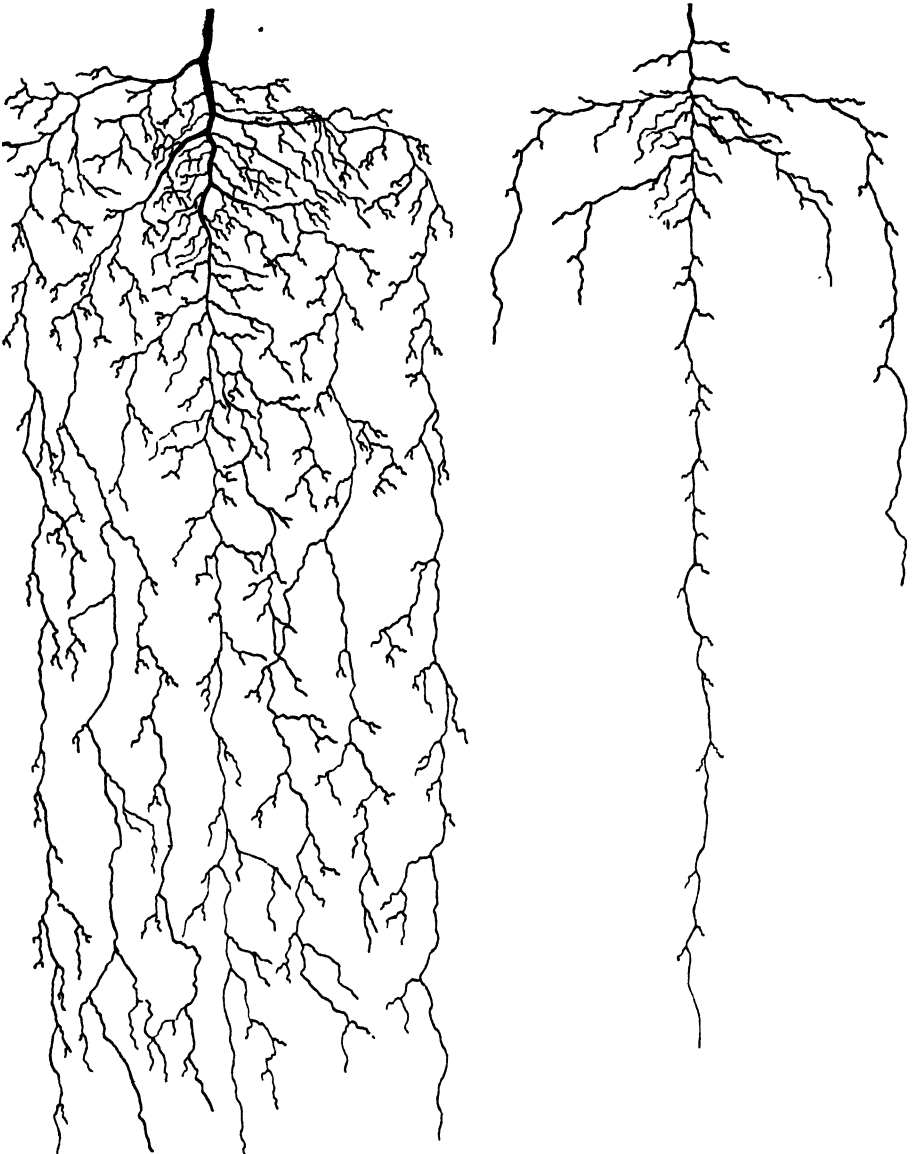


FIG. 2. Relative development of root systems of red clover under 15-hours (left) and 7-hours daily illumination.

branches of the long-day plants had developed many rootlets 5 to 10 cm. long and some reached a length of 36 cm. and extended to the bottom of the container. In fact, the surface 15 cm. of soil of the short-day container was much more poorly ramified with roots than the last 15 cm. of the other

container. Thus while the general plan of the root systems was the same in both cases, roots of the short-day plants, where growth of tops had been inhibited, represented a much earlier stage in development than those of the well developed plants in blossom (fig. 2).

RADISH

The radishes were in the cotyledon stage when placed under the two conditions. Within a single week the larger leaves and darker green color of the long-day plants were very noticeable and when the roots were examined at the end of a month marked differences were apparent (fig. 3). Although each of the ten plants possessed the same number of leaves, those of the long-day averaged just three times as large (1,365 sq. cm.) as those of the short-day plants.



FIG. 3. Average development of tops and fleshy portion of roots of long-day (right) and short-day plants of radish.

The radish is characterized by a rapidly growing and deeply penetrating taproot. Strong, profusely branched laterals arise in the surface 7.5 to 20 cm. of soil and constitute the major portion of the absorbing system, since at greater depths the taproot develops only short laterals.

The taproots of the smaller plants (fig. 3) were 20 to 75 cm. long, those of the larger ones reached greater depths and extended 10 to 15 cm. along the bottom of the container. The thread-like lateral roots and their almost cobwebby network of branches were of nearly equal abundance in both containers in the first 20 cm. of soil. But in the next 25-cm. layer this network of branches continued in the long-day plants while in the others it was absent and there were few branches except very close to the taproot. At 40 cm. depth, branches from the taproots averaged somewhat

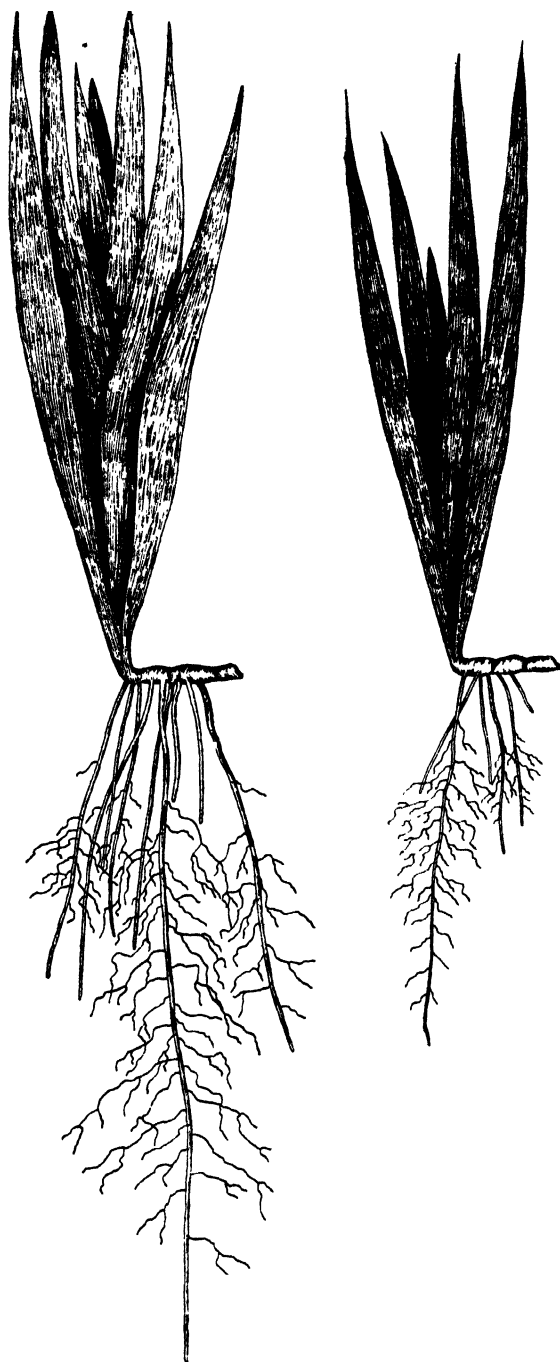


Fig. 4. Relative development of iris under long-day (left) and short-day illumination.

more than twice as long on the larger plants. Even the last 30-cm. layer of soil was penetrated at distant intervals by the vertical ends of these thread-like surface roots.

The fleshy portion of the short-day root system averaged 6 cm. long and 1.7 cm. in diameter; its volume was 58 cc. That of the larger plants was 12 cm. long and 2.7 cm. thick, and the volume 223 cc. Thus the root system of the long-day plants was fully twice as well developed as that of the short-day plants.

IRIS

The five rhizomes of iris placed in each of the two containers showed little differentiation of tops until near the time of root excavation on June 19. The average length of leaves of the long-day plants scarcely exceeded that of the short-day but the new leaves were 18 per cent. more abundant. They also averaged about 7 cm. longer and 0.4 cm. wider. The total dry weight of leaves was 2.7 times as great in the long-day plants.

The root system of the iris was very simple. It consisted merely of a few large roots which penetrated rather vertically downward and branched but sparingly. The length and diameter of these roots were carefully measured as they were excavated and counts of the number and measurements of the lengths of the branches were made. On the short-day plants the average number of main roots per rhizome was 6; on the long-day plants 9.2. The former were 1 to 2 mm. in diameter, the latter 1.5 to 3. The average lengths were 13.1 and 22.3 cm. respectively, and the maximum depth of penetration, in the same sequence, 30 and 58 cm. In the short-day container, roots were sparse below 20 cm.; the soil was well filled with roots of the long-day plants to 32 cm. in depth. The younger and shorter roots were unbranched. Primary laterals became more abundant and developed longer branches as the main roots grew longer. Hence, this portion of the absorbing system was much more fully developed in the long-day plants. For example, at 28 cm. depth short-day plants gave off branches only 1 to 4 cm. long, while 12 cm. deeper the larger plants had rootlets 4 to 9 cm. in length which were occasionally rebranched (fig. 4).

SUNFLOWER

Under the long-day illumination the sunflower rapidly developed from the cotyledon stage. The short-day plants were yellowish green and distinctly retarded in their development. They were not attenuated. When the roots were examined on June 17, the long-day plants had a height of 74 cm. and a stem diameter of 15 mm. Each possessed 16 leaves and the average leaf area was 1,924 sq. cm. (fig. 5). The smaller plants were 19 cm. tall and the stems 5 mm. thick. There were only 11 leaves per plant

and the average photosynthetic area was only 14 per cent. as great (270 sq. cm.).

The sunflower has a pronounced taproot with numerous strong lateral branches originating almost entirely in the first few inches of soil. The taproots of the short-day plants were 2 mm. in diameter at a depth of 4 cm.; those of the long-day plants were 12 mm. in diameter. The former



FIG. 5. Long-day and short-day plants of dahlia and sunflower about 6 weeks old. The larger plants grew under a long-day illumination. The containers are 80 cm. deep.

tapered to a width of only 1 mm. at 15 cm. depth but extended to the bottom of the container. Those of the long-day plants were 2 mm. thick at the 15 cm. level and 1 mm. in diameter where they grew along the bottom of the container. The largest branches of the short-day plants were 1 mm. or less in diameter and reached a depth of only 28 cm. Those of the long-day plants were 3 mm. thick, they were much more abundant, and some reached the bottom of the container which was covered with a fairly dense network of roots consisting of taproots and the long surface laterals, and the branches of both lots.

The working level of the primary branches (*i.e.* the depth to which most of the roots extended and to which depth maximum absorption occurred) was 15 cm. in the short-day plants, but 45 cm. in the others. The great mass of roots at the working level of the long-day plants, together with their greater diameters and longer and thicker branches, was in marked contrast to that of the short-day plants. The lateral roots originating below 20 cm. depth were 12 cm. or less in length in the smaller plants; on the larger ones, the secondary rootlets on the bottom of the container were of similar length and development. Below the working level, lateral roots were sparse in the short-day container; there was practically none beyond 55 cm. depth. In the long-day container they were more abundant and of larger diameter than were those of the short-day plants between 15 and 55 cm. depth. Even the last 15 cm. of soil contained numerous rootlets.

Thus the root development of the short-day sunflowers corresponded in every way with that of very much younger plants than those of the long-day which had made a normal development.

DAHLIA

The dahlias were about 5 inches tall and were developing a second pair of leaves when the one lot was subjected to the short-day. The two plants of each culture developed leaves at about the same rate but by the end of 15 days the long-day plants were twice as tall and the leaves of a darker green color. When taken off on June 17, the average length of stems was 71 and 26 cm. respectively and the diameter of the stems was almost twice as great in the taller plants. The larger plants had begun to branch and now had more and much larger leaves. The average relative leaf areas were 2,310 and 885 sq. cm. respectively (fig. 5).

The root system of the dahlia consisted of a number of main fibrous roots that arose from the base of the tuberous root. Some of these ran outward and upward, branching profusely to near the soil surface; and still others ran vertically downward. In addition, a few fibrous roots arose from the top of the tuberous root.

The number of roots arising from the base of the old, fleshy root was the same in both lots, varying individually from 10 to 39. They were better developed in the long-day plants where the largest roots were twice as thick, and the branches more numerous, larger, and longer. With a single exception, no roots occurred below 60 cm. on the short-day plants and the working level was 23 cm. The long-day plants extended their roots in considerable numbers quite to the bottom and sometimes along the floor of the container. The working level was nearly three times as great as that of the short-day lot and roots were much more abundant even in the deepest soil than below the 23 cm. level of the short-day plants. More-

over, the network of roots that extended above the bases of the tuberous roots was very much more pronounced in the soil of the long-day plants. Here also this network was found to continue to the working level at about 60 cm.

A lot of tuberous roots had developed from the base of the stems above the original propagule together with a few much-branched fibrous roots. The average number (35) was only slightly greater in the long-day plants where the fleshy roots were long and spindling (8.7×0.5 cm.) than in those of the short-day which were short and thick (5.4×1.6 cm.). The total volume of the latter (68 cc.) was over 2.5 times that of the long-day plants. While nearly all of the thickened but spindling roots ended without branching, the fleshy root continuations of the short, thick ones were branched, the laterals penetrating widely, and often to the working level.

ZIMMERMAN and HITCHCOCK (21) have shown that in certain varieties of dahlia, length of day determines the type of root system formed by cuttings. Heavy root storage is correlated with a short day and a fibrous root system with a long one.

RAGWEED

The ragweeds had a second pair of leaves at the beginning of the experiment. Within a week the long-day plants were twice as tall as those of the short-day, and, as in all other cases, the latter lacked the deep, dark-green color of the long-day lot. By June 27 when the experiment was concluded, the four long-day plants had made a vigorous vegetative growth and attained a height of 90 cm. but showed no signs of flowering. Those of the short-day were only 12 cm. tall and small inflorescences were beginning to appear. Average stem diameters were 10.6 and 4.1 mm. The leaves had three times the length and breadth in the larger plants and the dry weight of tops was 7.6 times greater in the long-day plants (fig. 6).

The root systems of the ragweeds showed almost as marked differences as the tops, those of the short-day plants being very poorly developed. The strong taproots of the larger plants were about 13 mm. in diameter; those of the smaller ones did not exceed 3.5 mm. Although the thread-like taproots of the latter extended to the bottom of the container and often ran 5 to 10 cm. along the bottom, they were poorly developed in comparison to the vigorous taproots of the larger plants, which undoubtedly would have extended two or three feet deeper.

Like the sunflower, this species gives rise to strong laterals in the surface 15 cm. of soil. In fact, these are so vigorous that they often equal the taproot in size and degree of branching. On the long-day plants they were frequently 4 mm. in diameter at their origin and many of them extended along the bottom of the container. In fact, they were larger throughout



FIG. 6. Development of great ragweed at the end of six weeks. The tall plants have had daily about 15 hours of light.

than the taproots of the short-day plants. Major branches of the short-day plants were only one-fourth as large, being mere threads which did not extend beyond 40 to 60 cm. in depth. Laterals, however, were fairly profuse but mostly short and only occasionally of the third order. Laterals on the major branches of the long-day plants were very much more abundant, several times as long, and often rebranched to the fourth order. Even in the center of the container, the secondary branches were as large as any of the primary branches of the taproots of the short-day plants. Smaller branches from the two lots of taproots varied in about the same proportion as those described.

Only in the surface 15 cm. of soil in the short-day containers were roots fairly numerous; at greater depths they were sparse, consisting of the thread-like taproots and their short, scattered branches. Conversely, al-

most every cubic centimeter of the entire soil of the long-day container was thoroughly ramified with roots, those in the upper half being especially dense, although great mats of roots also occupied the bottom layers of soil.

OATS

Because of an accident to one of the containers, it was necessary to replant the oats on June 3. After it was well established, the plants were thinned to 35 per container. Final measurements were made on July 19. The short-day plants were 32 cm. tall and in the fifth-leaf stage, the long-day plants were 53 cm. high, each had 6 or 7 leaves and on some the panicles were beginning to appear. The dry weight of tops was 4.4 and 11.4 grams respectively.

Oats develops a fine, fibrous root system. The primary root system consists (usually) of the three seminal roots (*i.e.* the primary root and two almost equally large laterals) and their branches. The secondary root system, which develops later and constitutes the larger part of the root mass, consists of numerous adventitious roots that develop from the lower nodes of the stem.

Among the short-day plants only a few roots of the primary system extended to the bottom of the container but practically all of those of the long-day not only reached this depth but also ran 10 to 15 cm. along the floor of the container. The latter were also much more profusely furnished with longer branches. Roots of the secondary system did not penetrate beyond 13 cm. in the short-day container, but they reached twice this depth on the long-day plants. Moreover, actual count showed that they were about 4 times as numerous and their branches were longer and well clothed with laterals. Thus the roots of the short-day oats corresponded to a much earlier stage of development than that shown by the long-day plants.

COSMOS

One lot of cosmos was placed under short-day illumination upon the appearance of the first leaves. Paler color and retarded growth were clearly evident a week later and by June 27 when the short-day plants were in full bloom, differences in vegetative growth were marked (fig. 7). The seven short-day plants averaged 35 cm. tall, with a stem diameter of only 2.5 mm. The other seven were 50 cm. high and the stems were 6 mm. thick. The dry weight of the stems, in the same sequence, was 1.4 and 10.0 grams, and that of the leaves 0.6 and 9.6 grams.

Cosmos has a taproot which penetrates deeply, and develops numerous strong laterals in the surface soil. The deeper portion of the taproot is well branched but the branches are relatively short.

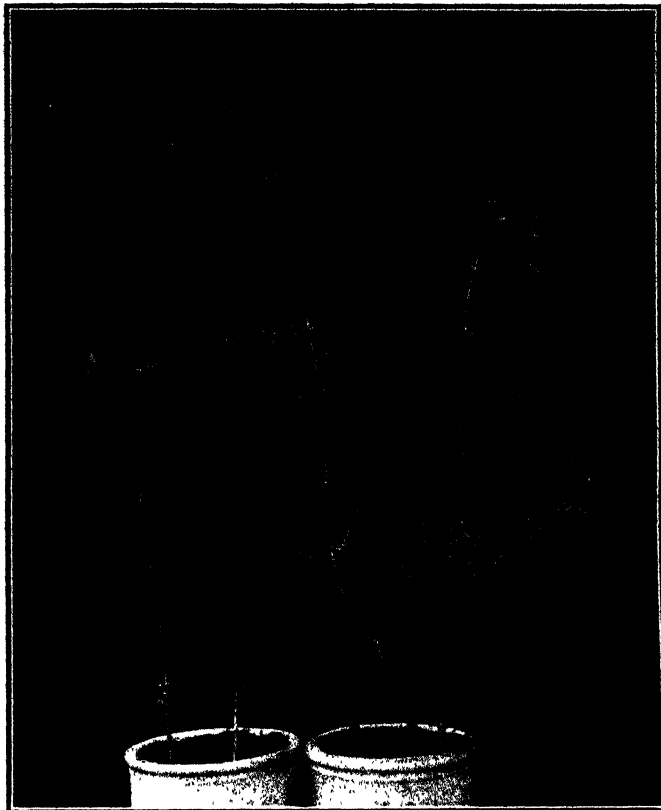


FIG. 7. *Cosmos* about 60 days old. Those receiving daily light for only 6 hours are in bloom. Two of the 7 plants from each container were cut and placed in the jars for photographing.

The main roots of the larger plants were 3 mm. in diameter, and except for the limited depth of soil would probably have extended to depths of 1.25 meters or more. The fine taproots of the smaller plants were no larger near their origin, than those of the larger ones at 80 cm. in depth. They were scarcely 75 cm. long. On the short-day plants 10 to 12 laterals barely 1 mm. thick arose in the surface 10 cm. of soil. The longest extended only 24 cm. and most of them were much shorter. Many of the main laterals were unbranched, others had branches a cm. or less in length. The long-day plants had approximately three times as many strong laterals, some originating at 15 cm. depth. These had twice the diameter and three times the number of laterals as those of the short-day. Moreover, the laterals were 1 to 5 cm. long and frequently rebranched. Branches from the tap-root were sparse and short below 35 cm. depth on the short-day plants.

But on the larger plants even at 50 cm. depth they were fairly abundant and some were 10 cm. long. The larger taproots branched throughout their length. The short, fine taproots of the smaller plants, the smaller number and limited extent of the branches, and the smaller number of branch orders all indicated an arrested development which corresponded in general with the lesser development of the tops.

CARBOHYDRATE DETERMINATIONS

The preceding data show that in every case the development of the absorbing system was clearly correlated with the transpiring surface. This was true for the long-day plants, *viz.*, clover, iris, and oats, which require long days for successful flowering and fruiting, as well as for cosmos, ragweed, and dahlia which come into blossom normally only when short days occur. The sunflower, which is not materially influenced in time of flowering by the length of day, at least in medium-high latitudes, likewise showed a clear correlation between development of root and shoot.

Where, as in this experiment, all conditions except that of length of day are favorable for growth, it would seem that development of both tops and roots would be directly correlated with the rate of food manufacture. This is clearly indicated by a series of measurements on the rate of carbohydrate manufacture in several of the species.

In these experiments, the photosynthetic activity is based on total carbohydrate content and upon increase in carbohydrate content of leaves after insolation, this being measured as reducing sugars. The photosynthetic activity was determined by the picric acid colorimetric method as modified by LONG (10, 11).¹

Photosynthetic determinations were made on the leaves of sunflower, radish, and dahlia on June 13 and 15; of the ragweed on June 13, 15, and 26; and of the red clover on June 26 and 27. All except June 27 were bright sunny days. Samples of the plants growing under long-day exposure were secured at daylight, 9:00 A. M., 4:00 P. M., and sunset. Those from plants under the short-day were taken at 9:00 A. M. and 4:00 P. M. In all instances, care was taken to secure samples from fully grown leaves of approximately the same age, that were shaded as little as possible.

Table I gives the actual amount of sugar per square centimeter in each sample, the relative amounts in the long- and short-day plants at 4:00 P. M., the increase in sugar content during the entire period of illumination, and the actual and relative increases between 9:00 A. M. and 4:00 P. M. for both sets of plants.

¹ These data are from unpublished work by Miss THEODORA KLOSE, to whom a detailed outline of the method was furnished by Doctor LONG.

TABLE I
PHOTOSYNTHETIC ACTIVITY OF LONG- AND SHORT-DAY PLANTS

		SUNFLOWER				RADISH				CLOVER			
		JUNE 13		JUNE 15		JUNE 13		JUNE 15		JUNE 26		JUNE 27	
		ACTUAL	RELA-TIVE	ACTUAL	RELA-TIVE	ACTUAL	RELA-TIVE	ACTUAL	RELA-TIVE	ACTUAL	RELA-TIVE	ACTUAL	RELA-TIVE
		mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.
Sugar per sq. cm.	Long day	Day-light 9 A. M. 4 P. M. Sunset	0.704 0.832 1.152 0.996	100	0.640 0.640 1.088 0.960	100	0.704 0.704 0.896 0.896	100	0.640 0.640 1.024 0.960	0.768 0.768 2.368 1.024	100	0.640 0.768 1.408 1.024	100
	Short day	9 A. M. 4 P. M.	0.640 0.768	67	0.640 0.896	82	0.704 0.768	86	0.640 0.960	0.384 0.372	37	0.423 0.646	46
	During entire day	Long day Short day	0.448 0.128		0.448 0.256		0.192 0.064		0.384 0.320	1.600 0.488		0.768 0.223	
	Between 9 A. M. & 4 P. M.	Long day Short day	0.320 0.128	100 40	0.448 0.256	100 57	0.192 0.064	100 33	0.384 0.320	1.600 0.488	100 31	0.640 0.223	100 35

TABLE I.—(Concluded)

		RAGWEED						DAHLIA					
		JUNE 13		JUNE 15		JUNE 26		JUNE 13		JUNE 15			
		ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE	ACTUAL	RELATIVE		
		mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.	mg.	per cent.
Sugar per sq. cm.	Day-light	0.768		0.640		0.512		1.024		0.768		0.768	
	9 A. M.	0.640		0.640		0.704		0.896		0.768		0.768	
	4 P. M.	1.024	100	1.088	100	0.992	100	1.152	100	1.280	100	1.280	100
	Sunset	1.088		1.024		0.896		1.024		1.280		1.280	
Increase per sq. cm.	Long day	0.704		0.640		0.576		0.768		0.640		0.640	
	Short day	1.152	113	1.152	106	0.896	90	1.024	89	1.152	90	1.152	90
	During entire day	0.448		0.448		0.480		0.256		0.512		0.512	
	Between 9 A. M. and 4 P. M.	0.448		0.512		0.320		0.256		0.512		0.512	
Increase per sq. cm.	Long day	0.384	100	0.448	100	0.288	100	0.256	100	0.512	100	0.512	100
	Short day	0.448	117	0.512	114	0.320	111	0.256	100	0.512	100	0.512	100

An examination of the table shows that on both June 13 and 15 the daily rate of increase in sugar in the sunflower was considerably higher in the long-day plants, and also that greater amounts of sugar per unit area occurred in the plants having the longer illumination.

Photosynthetic tests of the radish show, as in the sunflower, a much higher rate of daily increase in sugar content and also larger amounts of sugar per sq. cm. The differences are somewhat less on June 15, probably owing to differences in the place of selecting samples or in environment, but the decrease is consistent in the two species.

The red clover gave not only the greatest increase in photosynthate of any of the plants tested but also the greatest differences between the long- and short-day plants. Table I shows a much higher rate of increase in sugar content between 9:00 A. M. and 4:00 P. M., as well as during the entire period of illumination of the two lots of plants. The smaller amounts of photosynthate on June 27 are due to the fact that this was a cloudy day.

In contrast to the behavior of the three preceding species, ragweed and dahlia under long daily illumination gave no higher rates and, in fact, often lower ones, than those under 7-hour daily periods of light. The photosynthetic tests on the ragweed on June 13 showed a slightly higher rate of increase in the short-day plants between 9:00 A. M. and 4:00 P. M. The total daily increase during the entire period of illumination is, however, the same. The actual amounts of sugar at 4:00 P. M. are greater in the short-day plants than in those of the long-day. The tests on June 15 are essentially the same except that the total daily increase is greater in the short-day plants instead of being equal in the two sets as on June 13. On June 26 the rate of increase between 9:00 A. M. and 4:00 P. M. still remained higher in the short-day than in the long-day plants; contrary to the previous results, however, the total increase during the entire period of illumination of the long-day plants was greater than the increase during the 7-hour period of illumination of the short-day plants. The actual amount of sugar at 4:00 P. M., moreover, was greater in the former. The short-day plants were beginning to flower which, no doubt, resulted in rapid use of the photosynthate and perhaps accounts for the lower actual amount of sugar in these plants at this time. The rate of increase remained higher throughout in the short-day plants.

As regards the dahlia, table I shows a slightly higher amount of sugar at 4:00 P. M. in the leaves of the long-day plants than in those of the short-day. The rate of increase, however, between 9:00 A. M. and 4:00 P. M. is the same in the two sets of plants. The increase during the 7-hour period of light of the short-day is equal to the increase of the long-day plants during their entire period of illumination.

In interpreting these results it must be kept in mind that the rate of increase in these tests represents the balance between the process of photosynthesis on the one hand and those of respiration, translocation, and growth on the other. Hence, the higher rate obtained under certain conditions is not necessarily due to a higher rate of manufacture of materials under these conditions but may result in part from a slower rate of respiration, or removal, or growth.

GARNER and ALLARD have found that there are different optimal light periods for maximum vegetative growth, for sexual reproduction, and for tuberization, each of which varies with the species. The photosynthetic data given above seem to indicate that those plants, such as the radish and clover, whose optimal light period for reproduction lies above 12 hours (long-day plants) are more active photosynthetically or less active in removing or using the product under long-day illumination than under a 7-hour daily light exposure.

The growth data show that there was a rapidly increasing leaf area for photosynthesis and increased water loss in the clover, radish, and sunflower under long-day illumination. Notwithstanding the rapidity of growth and accompanying high rate of respiration for the necessary release of energy, the leaves of these plants showed the highest rate of increase in carbohydrates under equal periods of illumination. In addition, the extra hours of illumination resulted in an additional amount of food for increased growth. The demands for an ever increasing supply of water to replace that lost by transpiration, together with abundant materials for growth, resulted in every case in a root system the extent of which correlated well with the development of the tops. Under the short-day illumination less food was manufactured, growth of tops was retarded, and the root system was correspondingly abbreviated.

It seems that the dahlia and ragweed when grown under a long daily period of illumination, use the entire product of photosynthesis in promoting vegetative growth, the balance between the transpiring and absorbing system being well maintained. Under short-day illumination the dahlia accumulates a much greater relative amount of material in its fleshy roots. As pointed out by GARNER and ALLARD (4), this accumulation obviously indicates an excess of carbohydrates over current consumption. This surplus of carbohydrates is not due to increased photosynthetic activity but rather to the inability of the plant to utilize the carbohydrate, whether it be present in relatively large or small quantity. Since this portion of the food is not utilized by the growing plant, the result is a decreased extent of both root system and above-ground parts. The case of the ragweed is not quite so clear. It is a well established physiological fact, however, that respiration is high during the period of anthesis and much of the photo-

synthate furnished by the meager leaf area was undoubtedly used in supplying energy for this process. This interpretation is in agreement with that of GARNER and ALLARD (4) who conclude "that the duration of the daily illumination period not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material."

NIGHTINGALE (15a) has shown that radish, salvia, buckwheat, and soy beans were limited in the synthesis of nitrates to other forms of nitrogen by a 7-hour day. Associated with relatively little assimilation of nitrates was an accumulation of carbohydrates within these plants when they were subjected to short-day illumination. Carbohydrates accumulated in the short-day plants, presumably because there was relatively little utilization of them in the synthesis of nitrates to other forms of nitrogen.

Summary

The relative development of roots and tops of eight species of plants grown under 7-hour and 15-hour daily illumination respectively, was determined. The plants were grown for about 7 weeks in soil in containers 30 x 30 x 80 cm. in size, which permitted of rather normal root development.

Red clover, radish, iris, and oats, all long-day plants as regards flowering, developed large tops and proportionately extensive root systems when subjected to the 15-hour day. Under short-day illumination the growth of both tops and roots was greatly retarded and approximately to the same degree. Their development was similar to that of the long-day plants when the latter were only 3.5 weeks old.

Sunflower, whose time of flowering is less modified by the length of day, developed in a manner similar to the preceding species.

Dahlia, the great ragweed, and cosmos are short-day plants. They attained their greatest size and greatest root development under the 15-hour day. Under short-day illumination the dwarfed tops were furnished with a correspondingly meager absorbing system, although more food was accumulated in the short-day dahlia. Thus in all cases development of the root systems was in direct correlation with the development of tops.

The effect of length of daily illumination upon photosynthetic activity showed that red clover, radish, and sunflower are either more active in photosynthesis or less active in removing or using the product under long-day illumination than they are under a 7-hour day. Great ragweed and dahlia were found to be more or less equally active photosynthetically under the two light periods, or less active in removal or use of the products under short-day illumination.

LITERATURE CITED

1. AUSTIN, C. L. Influence of pruning upon top and root development. California Agr. Exp. Sta. Rept. 1923: 210. 1923.
2. CHANDLER, W. H. Some results as to the response of fruit trees to pruning. Proc. Amer. Soc. Hort. Sci. **16**: 88-101. 1919.
3. CRIST, J. W., and STOUT, G. J. Relation between top and root size in herbaceous plants. Plant Physiol. **4**: 63-85. 1929.
4. GARNER, W. W., and ALLARD, H. A. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871-920. 1923.
5. GERICKE, W. F. Investigations of physiological processes related to root systems of plants. California Agr. Exp. Sta. Rept. 1922. 116-117. 1922.
6. HARRIS, F. S. The effect of soil moisture, plant food, and age on the ratio of tops to roots in plants. Jour. Amer. Soc. Agron. **6**: 65-75. 1914.
7. JEAN, F. C., and WEAVER, J. E. Root behavior and crop yield under irrigation. Carnegie Inst. Washington Pub. no. 357. 1924.
8. JOHANSSON, NILS. Einige Versuche über die Einwirkung verschiedener Belichtung auf die vegetative Entwicklung von *Raphanus sativus* L. Flora **121**: 222-235. 1927.
- ✓ 9. LIVINGSTON, B. E. Note on the relation between growth of roots and of tops in wheat. Bot. Gaz. **41**: 139-143. 1906.
10. LONG, F. L., CLEMENTS, F. E., and HAUPT, C. R. Ecological measurement of photosynthate. Carnegie Inst. Washington, Year Book **24**: 322-323. 1925.
11. ————. Ecological measurement of photosynthate. Carnegie Inst. Washington, Year Book **25**: 352-354. 1926.
12. LUBIMENKO, V. N., and SZEGLOVA, O. A. L'Adaptation photopériodique des plantes. Rev. Gen. Bot. **40**: 675-689. 1928.
13. MAXIMOW, N. A., and LEBEDINCEV, E. Über den Einfluss von Beleuchtungsverhältnissen auf die Entwicklung des Wurzelsystems. Ber. deut. bot. Ges. **41**: 292-297. 1923.
14. ————, and KRASNOSSELSKY-MAXIMOW, T. Über den Einfluss von Beleuchtungsverhältnissen auf die Entwicklung und Tätigkeit des Wurzelsystems. Bull. Jard. Bot. République Russe **23**: 1-11. 1924.
15. MOELLER, H. Beiträge zur Kenntniss der Verzweigung. Land. Jahrb. **13**: 167-173. 1884.
- 15a. NIGHTINGALE, G. T. The chemical composition of plants in relation to photo-periodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. **74**. 1927.

16. PFEIFFER, N. E. Microchemical and morphological studies of effect of light on plants. *Bot. Gaz.* **81**: 173-195. 1926.
17. REID, M. E. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon. *Bot. Gaz.* **87**: 81-118. 1929.
18. TURNER, T. W. Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. *Amer. Jour. Bot.* **9**: 415-445. 1922. (See p. 442.)
19. WEAVER, J. E. Investigations on the root habits of plants. *Amer. Jour. Bot.* **12**: 502-509. 1925.
20. ———, KRAMER, J., and REED, M. Development of root and shoot of winter wheat under field environment. *Ecology* **5**: 26-50. 1924.
21. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Root formation and flowering of dahlia cuttings when subjected to different day lengths. *Bot. Gaz.* **87**: 1-13. 1929.

SOME SEASONAL CHANGES IN THE TRACHEAL SAP OF PEAR AND APRICOT TREES

F. G. ANDERSSSEN¹

(WITH SEVEN FIGURES)

The sap in the tracheae of plants has long been known to contain various substances derived from the soil or from the surrounding tissues. Most of our knowledge of its composition has been obtained by study of the exudate (bleeding sap) from wounds during late winter (bleeding season) (CLARK, 4; SCHROEDER, 23; HORNBERGER, 11; MOREAU and VINET, 16). This has limited the study to a relatively short portion of the annual cycle and to comparatively few plants. Qualitative studies such as those of FISCHER (9, 10) have shown that certain constituents, reducing sugars, occur in the tracheae at various times of the year. DIXON and ATKINS (6, 7) carried out the most comprehensive study on tracheal sap yet reported. They obtained sap from tracheae by centrifuging wood from various parts of trees at different times of the year. They were able to follow the trend of changes in total electrolytes and sugars throughout the year and to estimate the concentrations of the sugars. MACDOUGAL (13) has also reported a few observations on the sugar content of the sap of pines.

The principal obstacle to the study of tracheal sap has been the difficulty in obtaining the large quantities needed for quantitative work with such a dilute solution. The sap has an electrolyte content approximating that of tap water, and organic substances are usually equally low. The only methods of obtaining the sap outside of the bleeding season have been to centrifuge portions of wood as done by DIXON and ATKINS (6) or to wash out the contents of the tracheae with water as done by MACDOUGAL (13) and MASON and MASKELL (15). Neither of these methods is suited to obtaining large quantities of sap, and both are liable to give sap which has been altered by contamination. An improved method has been described by BENNETT, ANDERSSSEN, and MILAD (2) which reduces the liability of contamination and allows sap to be obtained from woody plants in quantities adequate for quantitative work.

It seems desirable to extend our knowledge of the composition and changes in the tracheal contents. The sap in the tracheae amounts to several per cent. of the weight of a woody stem. Our only accurate knowledge of it is limited to the bleeding season, during which period the tree is relatively inactive so far as the major activities of growth, absorption, trans-

¹ I take pleasure in expressing my grateful appreciation to Dr. J. P. BENNETT for his interest and valuable advice throughout this work.

piration, food manufacture, and translocation are concerned. Exact knowledge of the composition of, and changes occurring in, the tracheal sap, may aid materially in understanding these activities. The work reported here is a preliminary study of tracheal sap from pear and apricot trees. The sap was obtained by the gas displacement method (2) from the main branches of three-year-old Bartlett pear and Royal apricot trees growing in a heavy clay-loam soil. Each branch extracted consisted of two- and three-year-old wood. Extraction was always done within two hours after cutting the branches, it having been found that appreciable changes occurred in the sap if the branches were cut several hours before use. The branches were cut the same time of the day in order to avoid possible diurnal changes in the sap. Appreciable diurnal variation was noted by HORNBERGER (11) in the titratable acidity of sap from bleeding Birch trees. The sap obtained in the present work was colorless and clear or slightly cloudy, and of relatively uniform conductivity throughout the length of each branch.

Expressed sap, used for comparison with tracheal sap in part of the work, was obtained from portions of the same branches used for extraction of tracheal sap by grinding the wood after removal of the bark and pressing the ground tissue under a pressure of 400 kg. per sq. cm. The expressed sap thus obtained was clear but highly colored.

Buffer value and reaction of sap

Titration curves shown in figure 1 were prepared for tracheal and expressed sap of the Bartlett pear. Fresh 5-ml. portions extracted May 16 were titrated with 0.02 N H_2SO_4 . The reaction of tracheal sap was determined colorimetrically, that of expressed sap electrometrically. From the curves it may be seen that the buffer value of expressed sap was about 25 times that of tracheal sap at the middle of May. The buffer value of both would doubtless vary during the year.

The low buffering of tracheal sap shows that extreme care must be exercised in determining its reaction. Contamination during extraction with very small amounts of vacuolar sap or other cell contents, or losses of dissolved gases might markedly affect it. Especial care was taken that all contamination from the cut surface of the wood through which the sap emerged was washed away before samples were retained for use. This was accomplished by determining the conductivity of succeeding ml.-portions of the sap as it was collected and discarding these until the conductivity became uniform. In the gas displacement method the sap is collected in a partially evacuated vessel, causing a loss of part of the dissolved gases. The gas of chief concern in relation to the reaction of the sap is carbon dioxide. This is known to be present in considerable amounts in the gases in the tracheae

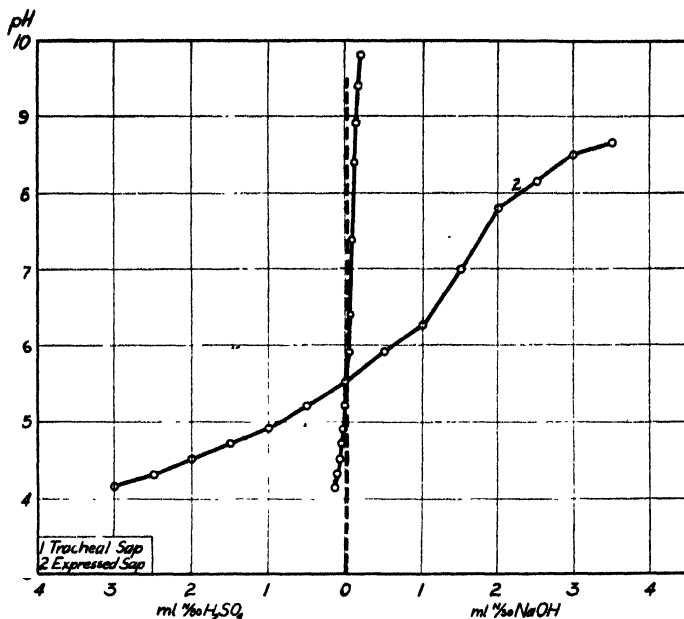


FIG. 1. Titration curves of sap of pear branches at the middle of spring.

(MACDOUGAL, 13; CLARK, 4) and tracheal sap would consequently be rich in it. It was not feasible in the present work to attempt to collect the sap with its full carbon dioxide content, and the losses during extraction would be variable. In order to make the samples comparable they were allowed to come into equilibrium with the air. The reaction of the sap as determined was then not necessarily the same as it was within the tracheae; but the variations in reaction found were due to substances other than carbon dioxide.

The expressed sap with its relatively high buffer value presented no especial difficulties in preparation.

The reaction of tracheal and of expressed sap from pear and apricot branches was determined at monthly intervals throughout a year. For each reported value sap was extracted separately from six branches, each from a different tree, the reactions determined separately and averaged. The results are shown in figures 2 and 3. The outstanding features of the curves for both apricot and pear is their convergence in the late winter and wide divergence later. For the pear, fig. 2, the period of approach lasts through spring, summer, and fall. During this period the curves are almost parallel. For the apricot the divergence of the curves began in midsummer and the period of close approach is relatively short. During one-half of the year in the apricot and one-third in the pear the tracheal sap was con-

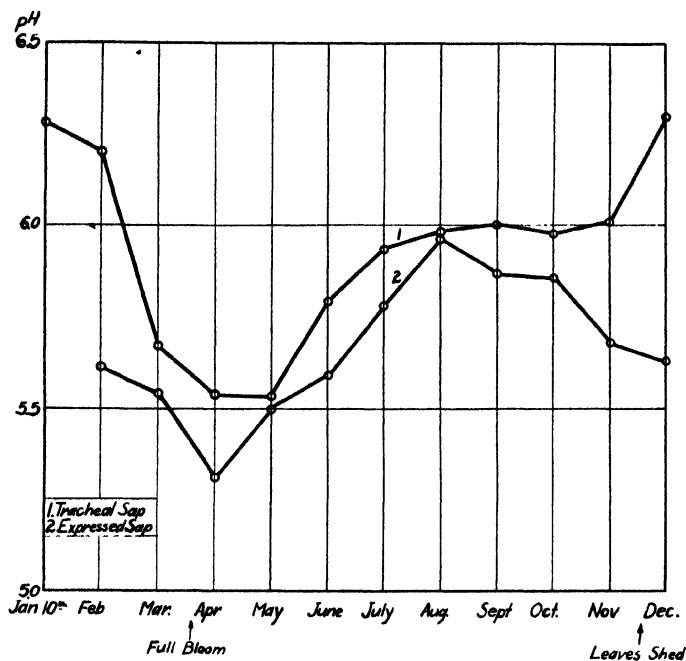


FIG. 2. Reaction of sap from pear branches.

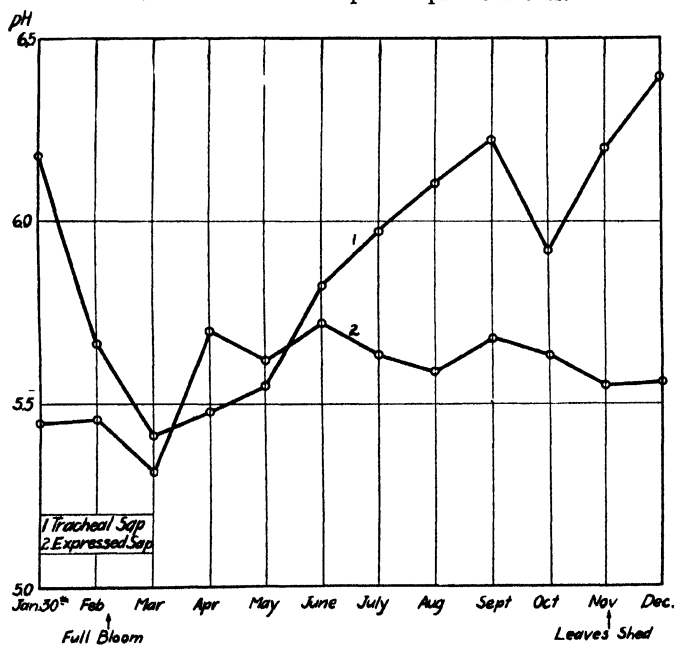


FIG. 3. Reaction of sap from apricot branches.

siderably more alkaline than the expressed sap. The maximum difference in reaction was in both cases about three-fourths of one pH. The total fluctuation in reaction was about 0.6 pH in the expressed sap of the pear and 0.7 pH in the tracheal sap, while in the apricot the expressed sap changed only about 0.4 pH and the tracheal sap about 1.0 pH. The winter divergence in the reaction was then due about equally to changes in expressed and tracheal sap in the pear, while in the apricot it was due more largely to changes in the tracheal sap. The trends of the curves suggest that during the active growing season the reaction of the tracheal sap is strongly influenced by that of the surrounding tissues, while during the dormant season this influence is absent. It seems probable that the increased acidity in early spring is due to an increase in organic acids in the sap. During the period of renewed growth respiration is very active and may result in a general increase in the concentrations of organic acids present in the tissues. HORNBERGER (11) found that the titratable acid in "bleeding" sap increased during the blossoming period and later decreased.

Inorganic constituents

It has long been known that the tracheal sap obtained from bleeding plants contained salts. SACHS (22) detected the presence of K, Ca, PO_4 , and SO_4 . HORNBERGER (11) studied quantitatively the exuded sap of the Birch and Hornbeam and determined the amounts of Na, Ca, and Mg present. PFEFFER (18) pointed out that the composition of the sap is not constant. The study of bleeding sap, of course, gives a very incomplete picture, leaving the composition and changes in the sap during most of the year entirely unknown. The most comprehensive investigation of tracheal sap is that of DIXON and ATKINS (6, 7) mentioned previously. The contamination of the sap by materials from the injured cells at the cut surface of the branch during the extraction of tracheal sap by the centrifuge method makes it seem probable that the results of DIXON and ATKINS are somewhat too high.

TOTAL ELECTROLYTES

The specific resistance of the sap was determined at monthly intervals throughout the year. The sap used was of the same lots as used in the determinations of reaction. Each value reported was the average of six determinations, each on sap from a single branch. The results are presented in figures 4 and 5. Both kinds of trees showed a minimum concentration of electrolytes during the dormant season, the pears at about midwinter and the apricots at the time of leaf fall. From very early spring there was a very rapid increase in concentration, reaching a maximum at or shortly after full bloom; after this maximum there was again a gradual decrease in

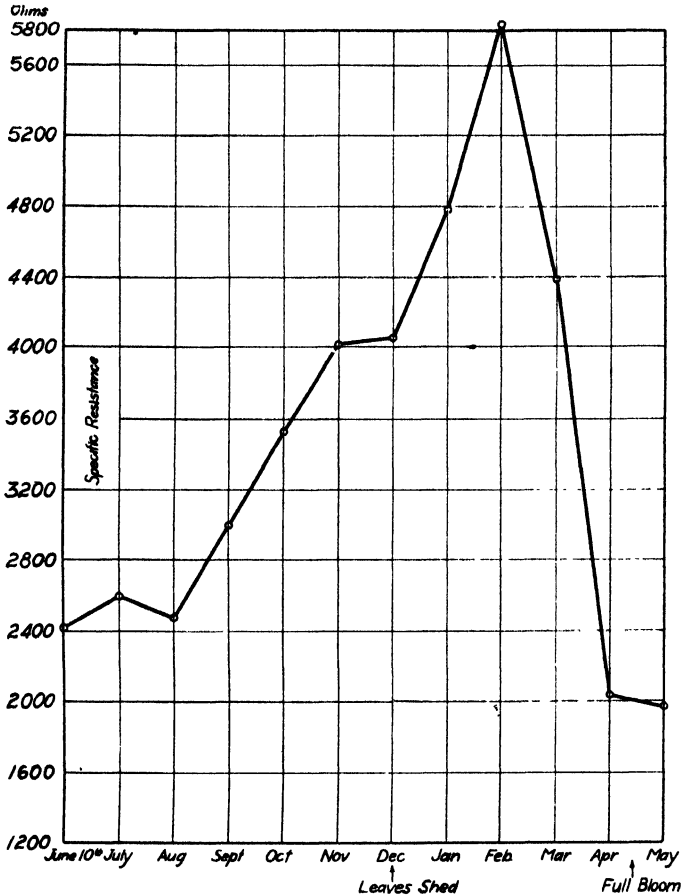


FIG. 4. Specific resistance of tracheal sap from pear branches.

concentration through the summer. The results in general agree with those of DIXON and ATKINS (6, 7) and of SCHROEDER (23).

The rapid increase in concentration in early spring may have been due to the action of one or more of several factors: the increase occurred at a time when the differentiation of tracheae was beginning, and the contents of such cells became part of the transpiration stream; at the same time the rate of respiration was increasing and the resultant hydrolysis and oxidation of cell constituents may have increased the concentration of diffusible ions in the parenchyma surrounding the tracheae, thus favoring the diffusion of more ions into the vessels. It is also possible that the permeability of the protoplasts may have increased during this period of increased activity, allowing an influx of solutes from the concentrated sap of the parenchyma cells into the tracheae. The roots were also actively growing at this

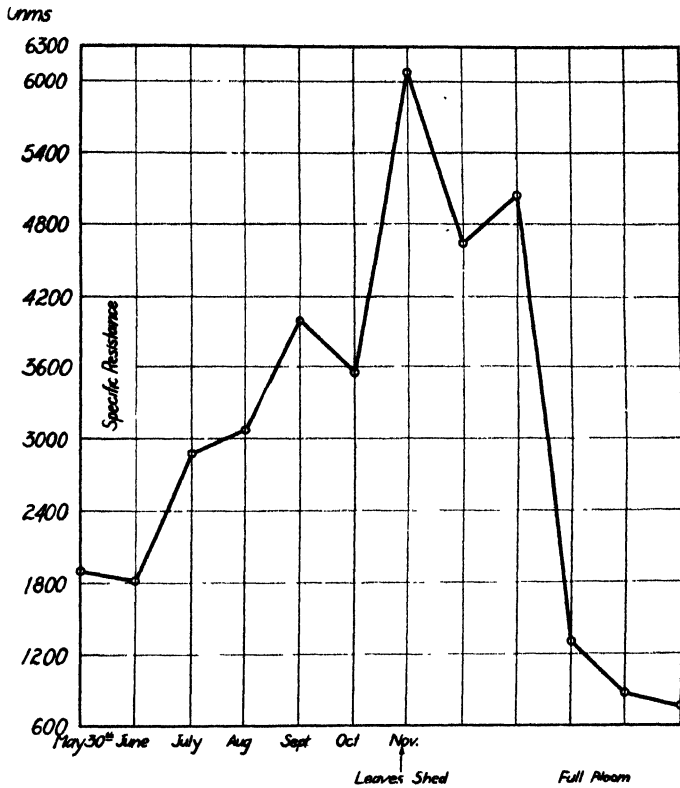


Fig. 5. Specific resistance of tracheal sap from apricot branches.

period, and a rapid absorption of ions from the soil solution would presumably be reflected in the concentration of the tracheal sap.

At or shortly after the blossoming season the leaves began to develop, and transpiration became more and more rapid. This would apparently cause a relatively greater intake of water than of salts, and result in a large dilution of the electrolytes in the transpiration stream. During the summer a decrease of root activity would further reduce the salt intake while the transpiration remained large. The decrease in concentration of electrolytes may be found to continue into early winter, if the tracheae have not yet reached their maximum water content by the time all the leaves have been shed.

The irregularities in the curves seem to be related, to a certain extent, to moisture conditions of the atmosphere and the soil. During rainy weather or just after an irrigation the concentration of the tracheal sap was generally less than during a dry period. Temperature changes also may exert some effect in this respect; MOREAU and VINET (16), working with

bleeding sap of the vine, found a fall in temperature to be followed by a decided increase in concentration of the sap.

INDIVIDUAL INORGANIC CONSTITUENTS

These were determined in the tracheal sap of the pear only at two times in the year at approximately the points of lowest and highest total concentration of electrolytes as indicated by the curves. Large amounts of tracheal sap were collected from pear branches on November 10, and on May 10 following.

The methods used in analyzing the sap are briefly indicated in the following:

Ca, Mg, and K were determined on the same sample, using 150–200 ml., depending upon the season. The Ca was determined as calcium oxalate, the Mg as MgNH_4PO_4 and the K as K_2PtCl_6 .

The sulphate was determined as BaSO_4 in 100–150-ml., and the Cl as AgCl in 150–200-ml. samples. Phosphate was determined by a modification of the colorimetric method of ATKINS (1) in 0.2–25.0-ml. samples. For Fe a slight modification of the colorimetric method of MARRIOTT and WOLF (14) was used on 50–100-ml. samples.

An analysis of a 1:1 water extract of the soil on November 10 at 18 inches and at 36 inches deep was made by the same methods. The results are shown in table I.

TABLE I

INORGANIC CONSTITUENTS IN TRACHEAL SAP OF PEAR AND IN THE SOIL EXTRACT

	DEPTH	SOIL EXTRACT NOVEMBER 10	TRACHEAL SAP NOVEMBER 10	TRACHEAL SAP MAY 10
	<i>inches</i>	<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>
Ca	18	18.0	16.6	84.7
	36	7.5		
Mg	18	7.2	0.8	23.5
	36	5.4		
K	18	140.9	23.6	59.6
	36	230.0		
Fe	18	1.8	1.0	2.1
	36	1.0		
SO_4	18	37.2	8.3	31.8
	36	30.0		
Cl	18	14.0	3.2	4.5
	36	9.5		
PO_4	18	1.0	10.6	25.2
	36	trace		

Without exception there was a very marked increase in concentration between November and May of the substances determined. Several of the ions on May 10, Ca, Mg, and PO_4 , even far exceeded the concentration in the soil extract. Unfortunately a soil extract on May 10 was not obtained. Probably the concentration of the soil extract in the spring was higher than in November, but whether the difference would be closely related to the difference in sap concentration remains to be determined.

In the case of phosphate there appears to be an immediate source other than the soil for the relatively large amount found in the sap in the spring. The results of phosphate determinations at monthly intervals are shown in table II. The phosphate content in pear sap increased to fifteen fold between February 10 and May 10. This relatively large increase in concentration of an ion relatively low in concentration in the soil suggested the possibility that much of the phosphate present in the sap may have come from the surrounding tissues. This suggestion was more strongly supported by examination of apricot sap in which the increase from January 3 to March 30 was 171 times.

TABLE II
PHOSPHATE CONTENT OF TRACHEAL SAP

DATE	PEAR	DATE	APRICOT
	<i>ppm.</i>		<i>ppm.</i>
Aug. 10	12.7		
Sept. "	11.6		
Oct. "	6.9		
Nov. "	10.4		
Dec. "	2.3		
Jan. "	1.8	Jan. 3	0.9
Feb. "	1.8	Jan. 31	10.7
Mar. "	1.7	Mar. 1	74.0
Apr. "	22.5	Mar. 30	154.0
May "	25.2	Apr. 30	37.5

It is definitely known that phosphorus is utilized during the synthesis of starch, proteins and perhaps other complex substances, and that on hydrolysis of such substances the phosphorus may again be set free as phosphate. NORTHROP and NELSON (17), making a quantitative study on phosphorus content of starch, showed the presence of 0.6 per cent. of phosphorus, 95-97 per cent. of which is in organic combination.

Further evidence favorable to the view that the above increase in phosphate may be due, at least in part, to release from an organic source was found by CAMERON (3) in some carbohydrate studies on citrus trees; the

evidence seemed conclusive that as the amount of starch in the wood increased through the season the amount of PO_4 extractable with water decreased. It seems probable, then, that part of the phosphate content of the tracheal sap may have come from the surrounding cells in which stored starch was being hydrolyzed in the late winter.

Organic constituents

FREE REDUCING SUBSTANCES AND SUGARS

Determinations of the reducing power, before and after inversion, of the sap from the pear were made on the same lots of sap used in following the total electrolytes. Each point on the curves represents the average of six determinations. The picric acid colorimetric method essentially as described by WILLAMAN and DAVISON (25) was used in determining reducing power. The method was found to give very consistent results, and was well adapted to this work because only 2 ml. of sap was required for each determination. The results are shown in figure 6. The designation "free reducing substances" is used instead of sugars, because it appears highly probable that not all the reducing substances present were sugars; but sucrose appears to be an accurate designation for the inversion product, because the same values were obtained when hydrolyzing with picric acid according to the cited method (25), as with invertase.

The most striking fact shown by the curves is the very marked increase in both reducing substances and sucrose shown during very early spring. Then the sucrose rapidly fell to practically zero shortly after full bloom. The reducing substances continued to increase at first, seemingly at the expense of the sucrose, up to a maximum at full bloom, and fell exceedingly rapidly to zero within the next month. There was a period of two to three months in summer during which the sugars were present, if at all, in indeterminable amounts. In early fall there again appeared a moderate amount of reducing substances and a small amount of sucrose. From this time on both the curves slowly rose through winter until they again reached the vernal maximum.

DIXON and ATKINS (6, 7) also report finding a great increase in sugar content in early spring. In the deciduous and in the evergreen trees used by them, the sucrose more often exceeded the reducing substances. Their reducing substances at the vernal maximum consisted of hexoses and maltose; later in the season the maltose was absent. FISCHER (9, 10), using microchemical methods, found a similar accumulation of reducing substances in the tracheae at the time of blossoming. SCHROEDER (23) found in the exudate from a tapped Birch tree within a period of seven weeks from early to late spring, a great decrease in concentration of sugars.

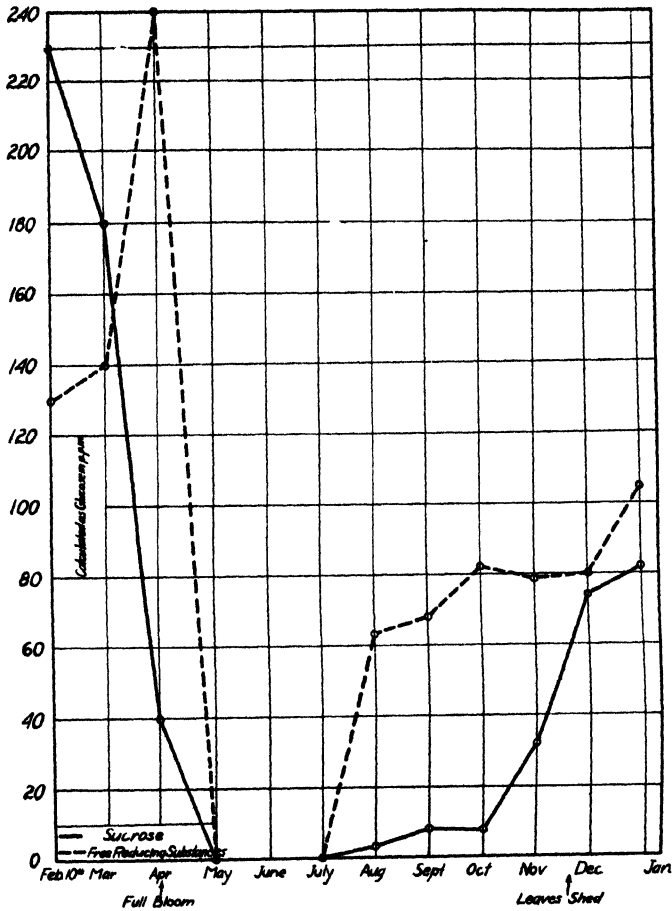


FIG. 6. Free reducing substances and sucrose in tracheal sap from pear branches.

MACDOUGAL (13) reports the presence of appreciable amounts of reducing substances in sap extracted from the wood of Monterey Pine. The method of obtaining sap was essentially that of water displacement described by BENNETT, ANDERSEN, and MILAD (2). An aqueous solution of dye was forced through the stem under a pressure of less than one atmosphere. The extractions reported were from June to October and show quite wide variations in sugar content. Since no mention is made of precautions taken to exclude such sources of error as mixture with the displacing water which may forge ahead of the dye (EWART, 8; PRIESTLEY and ARMSTEAD, 20), or with substances from injured cells around the point of extraction (BENNETT, ANDERSEN, and MILAD, 2), it is not clear that the variation in sugar content reported is not in part a result of the conditions of extraction. A

similar result would arise in extraction of sap by the centrifuge method of DIXON and ATKINS (6) and in the obtaining of "bleeding" sap.

PRIESTLEY (19) and PRIESTLEY and WORMALL (21) considered it wrong to suppose that the great increase in sugar in the trachea in early spring arose directly from the hydrolysis of starch; they believed that it came from the contents of cells differentiating into vessels. However, the great increase in sugar content found in the present work took place so early in the spring that one can scarcely consider differentiation to have been rapid enough to account for it. Furthermore, in late spring differentiation of cells into vessels was undoubtedly taking place much more rapidly, so that, even though the transpiring surface and the rate of the transpiration stream had simultaneously increased, one might have expected at least determinable amounts of sugars from this source to be found in the tracheal sap. It has also been determined that the time when the sugars drop to a minimum is likewise the time when the starch has been completely hydrolyzed and has disappeared from the more active outer section of the wood (10). It seems that, in the pear, the sugar content of the tracheal sap bears a close relation to the disappearance of starch.

NITROGENOUS CONSTITUENTS

The total nitrogen present in the tracheal sap was found to consist almost entirely of organic nitrogen. Nitrate could be detected with the diphenylamine reagent only after concentration of the sap. On November 10 the sap contained 60 ppm. of total nitrogen, and on May 10 163 ppm. of which 110 ppm. were present as amino nitrogen by VAN SLIKE's method and 49 ppm. as amide by the method of SACHSSE. There was apparently very little or no protein present. SCHROEDER (23), however, found as high as 33 ppm. of heat-coagulable protein in the sap of the Birch in April.

TOTAL SOLIDS

The usual Beckmann apparatus was found unsatisfactory for determining total concentration of solutes on account of the dilution of the sap. Determinations of refractive index at 25° C. were made with an Abbé's refractometer on the same sap as was used for electrical conductivity measurements. The data are represented in figure 7. It could not be expected to correlate these curves for total solids with those for total electrolytes, reducing substances and sucrose, because there are apparently other substances present for which analyses were not made. Thus, it has been found in the sap bleeding from a vine that the amount of organic acids present exceeds the total amount of all other organic substances (21). The higher values of refractive indices during the winter months coincide roughly with the increased amounts of organic and inorganic constituents

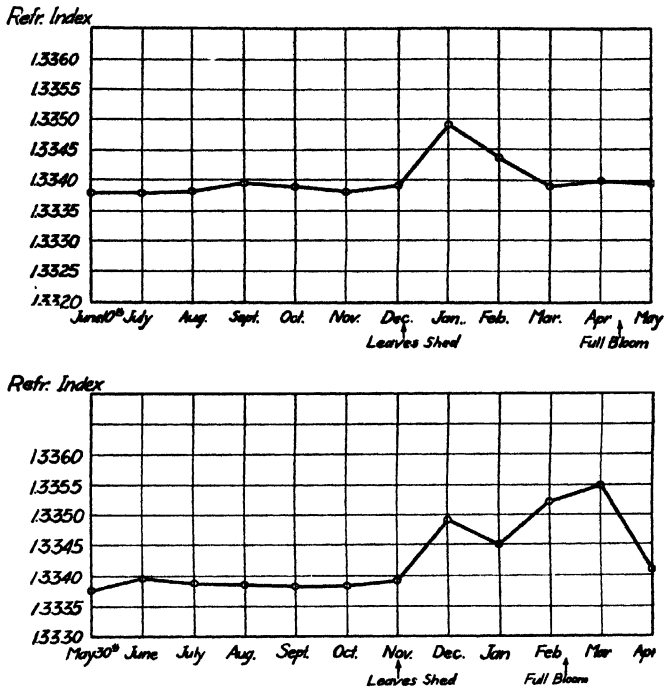


FIG. 7. Refractive indices of tracheal sap from pear (upper curve) and apricot branches.

found in the sap during that period. But the general lack of parallelism between the curves for refractive indices and those for total electrolytes, reducing substances and sucrose indicate that other substances than these were affecting the results.

It is not possible to compare these results directly with those obtained for other deciduous trees, *Acer macrophyllum* and *Ulmus campestris*, by DIXON and ATKINS (6, 7), since they determined the freezing point depression of the sap which is dependent upon the total solutes. They report a low osmotic concentration during late autumn and winter, whereas the pear and apricot both showed an increase in total solids in winter. This may be a difference shown by the different plants used, or it may quite possibly be due to the methods employed.

Radial distribution of sap constituents

STRASBURGER (24) and later DIXON (5) pointed out that the interpretation so often given to ringing experiments rests on the assumption that it

is possible to ring a branch without causing injury to the tracheae of the ring of wood adjoining the cambium and bark. Even though the ring of bark may be removed without directly injuring the tracheae, the wounding and exposure to the air is sufficient to cause injury, resulting in the clogging of such tracheae with air and gums. Furthermore, the outer tracheae are considered to be the most important in the transport of solutes up the tree, so that interference with the flow through these would be sufficient to retard or prohibit growth of buds above the ring, or to cause the starvation of the tree as a whole when the trunk was ringed. Because of the divergence of opinion on this matter, it was considered of interest to actually determine the difference in concentration of the sap collected from the outer tracheae and of that from the inner tracheae.

The method of the extraction of the sap is the same as that referred to earlier (2). When the branch was prepared as described there, a sharp-edged metal tube, about 1 inch long, was driven into the peeled end of the branch. The tube selected was of such a diameter as to include all of the cross-sectional area of the branch except the outer annular ring. During the extraction, the sap from the inner rings of wood was led by the metal tube into one collecting vessel while that from the outer ring dropped directly into another vessel. As in the previous extractions, the first few cubic centimeters collected in the tubes were discarded on account of contamination. A few of the results obtained by this method on pear branches are shown in table III.

TABLE III
RADIAL DISTRIBUTION OF SAP CONSTITUENTS IN THE PEAR

DATE	pH		FREE REDUCING SUBSTANCES		TOTAL REDUCING SUBSTANCES AFTER HYDROLYSIS		SPECIFIC RESISTANCE	
	OUTER	INNER	OUTER	INNER	OUTER	INNER	OUTER	INNER
			<i>ppm.</i>		<i>ppm.</i>		<i>Ohms.</i>	<i>Ohms.</i>
Feb. 24, '27	6.3	6.6	180	none	350	trace	4320	6550
	6.2	6.5	160	none	450	trace	3590	9520
April 4, '27	5.9	6.2			409	trace	2370	5000
	5.8	6.1			500	trace	2240	5030
May 9, '27	5.3	5.5	132	none	409	none	1615	2420
	5.3	5.5	200	none	240	none	1640	2380

Branches 3 years old were used for the results reported in the table in order to obtain large amounts of sap. However, similar results were obtained when one-year-old shoots of the previous season's growth were used. In this latter case the shoots had a diameter of 1 to 1.5 centimeters, and the

area enclosed by the metal tube comprised about half of the cross-sectional area of the shoot.

Nitrogen determinations were also made on outer and inner tracheal sap from 3-year-old branches on April 4. The outer sap contained 65.5 ppm. while the inner sap contained 26 ppm. of total nitrogen.

The specific resistances in table III show very definitely that the sap in the outer tracheae is much more concentrated in electrolytes than that in the inner tracheae. The concentration of the sap obtained from the inner or outer tracheae by the above method will undoubtedly depend upon the fraction of the whole cross-section which is being considered as inner or outer tracheae. This may possibly account for the differences found among different branches taken on the same date.

MACDOUGAL (13) reports differences in the sugar content of sap extracted from the different annual rings of the Monterey Pine. The results reported in this paper are in general agreement with those of MACDOUGAL; a more concentrated sap was obtained from outer than from inner annual rings. MACDOUGAL's extractions were made from June to October while the pear saps were extracted from February to May. While too few data are available for comparison of these results, it may be briefly pointed out that the sugar concentrations found by MACDOUGAL in pine sap were usually very much higher than those found in pear sap, and that a much wider variation of concentrations was reported for the former. It does not seem probable that pine sap in the summer season would be so much more concentrated in sugar and free reducing substances than pear sap in the spring. DIXON and ATKINS (7) did not find any greater concentrations of sugar in the tracheal sap of evergreens than of deciduous trees. It is suggested that much of the reducing substances found by MACDOUGAL came from the injured living cells adjoining the bored hole through which the sap was extracted. As pointed out by BENNETT, ANDERSEN, and MILAD (2) this injured surface has a very marked effect on its sugar content.

With regard to the sugar and free reducing substances it may be seen that the outer tracheae contained practically all of these throughout the period of observation. When all the sugar was found in the outer tracheae during early spring, it was thought possible that the situation might become reversed in late spring, when all the starch in the outer ring would have been hydrolyzed and the starch from the inner rings would be rapidly hydrolyzing. This was not found to be the case; on May 9 sugar was found only in the outer tracheae after starch had completely disappeared from the outer ring of wood. This fact seems to show that the starch in the inner rings, when hydrolyzed, does not pass into the adjacent tracheae but is

transferred along the medullary rays towards the outer tracheae into which it is delivered.

The sap from the outer tracheae is also invariably more acid than that from the inner. This seems probably related to the fact that the outer tissues are the younger and more active metabolically and are close to the very active cambium and layers of differentiating cells.

Summary

1. The preliminary data reported in this paper include determinations of buffer value, reaction, content of electrolytes, individual inorganic constituents, free reducing substances, sucrose, nitrogenous compounds, and total solids in tracheal sap of pear or apricot branches at various times during the year. The radial distribution in tracheal sap of acidity, total electrolytes, and free reducing substances before and after inversion is also shown for pear branches during the early spring.

2. The buffer value of tracheal sap at the middle of spring was found to be about one twenty-fifth that of expressed sap from the same tissues.

3. Tracheal and expressed sap reached maximum acidity in early spring, then fell gradually to minimum acidity in winter. The change from minimum to maximum acidity in early spring was very rapid and coincided with the resumption of growth.

4. Total electrolyte concentration and increased acidity of the tracheal sap ran parallel. The lowest concentration occurred in winter, followed by a very rapid increase in early spring, at the time of growth resumption. A gradual decrease took place during the summer and fall.

5. Individual inorganic constituents in the tracheal sap showed a large increase in concentration from early winter to early spring.

6. Phosphate in the tracheal sap showed a very large increase between late winter and early spring. It is suggested that this increase is due in large part to phosphate set free in the hydrolysis of starch.

7. Free reducing substances and sucrose showed a high concentration in late winter and early spring, a rapid decrease in late spring, an indeterminate low amount during summer, and a gradual rise during fall and winter.

8. The total solids, determined refractometrically, were found to rise during the winter months and decline during the spring, and then remain at a relatively uniform level during the remainder of the year.

9. The free reducing substances and sucrose were found to be limited to the outer annual ring in three-year-old pear branches during late winter and spring. Total electrolytes were about twice as concentrated in the outer

annual ring as in the inner rings. The sap from the outer annual ring was more acid than that from the inner rings.

DIVISION OF POMOLOGY,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. ATKINS, W. R. G. The rapid determination of available phosphate in soil by the coeruleo-molybdate reaction of Denigès. *Jour. Agr. Sci.* **14**: 192-197. 1924.
2. BENNETT, J. P., ANDERSEN, F. G., and MILAD, Y. Methods of obtaining tracheal sap from woody plants. *New Phytol.* **26**: 316-323. 1927.
3. CAMERON, S. H. Unpublished data.
4. CLARK, W. S. Observations upon the phenomena of plant life. *Massachusetts State Board of Agr. Ann. Rept.* **22**: 204-314. 1875.
5. DIXON, H. H. The transpiration stream. Pp. 52-53. Univ. of London Press. 1924.
6. ———, and ATKINS, W. R. G. On the constituents and concentration of the sap in the conducting tracts, and on the circulation of carbohydrates in plants. *Sci. Proc. Roy. Dub. Soc. n.s.* **14**: 374-392. 1915.
7. ———. On the composition of the sap in the conducting tracts of trees at different levels and at different seasons of the year. *Sci. Proc. Roy. Dub. Soc. n.s.* **15**: 51. 1916.
8. EWART, A. J. On the ascent of water in trees. *Phil. Trans. Roy. Soc. London B* **198**: 41-85. [1904] 1906; and *B* **199**: 341-392. [1907] 1908.
9. FISCHER, A. Glycose als Reservestoff der Laubhölzer. *Bot. Zeitg.* **46**: 405-417. 1888.
10. ———. Beiträge zur Physiologie der Holzgewächse. *Jahrb. wiss. Bot.* **22**: 73-160. 1890.
11. HORNBERGER, R. Beobachtungen über den Frühjahrssaft der Birke und Hainbuche. *Forstliche Blätter.* **1887**: 321; abstr. in *Bot. Centralbl.* **33**: 227-229. 1888.
12. MACDOUGAL, D. T. Reversible variations in volume, pressure, and movements of sap in trees. *Carnegie Inst. Washington, Pub. no.* 365. 1925.
13. ———. The hydrostatic system of trees. *Carnegie Inst. Washington, Pub. no.* 373. 1926.
14. MARRIOTT, W., and WOLF, C. G. L. The determination of small quantities of iron. *Jour. Biol. Chem.* **1**: 451-461. 1906.

15. MASON, T. G., and MASKELL, E. J. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. *Ann. Bot.* **42**: 189–253. 1928.
16. MOREAU, L., and VINET, E. Sur la composition des pleurs de vigne. *C. R. Acad. Agr. France* **9**: 554–557. 1923.
17. NORTHROP, J. H., and NELSON, J. M. The phosphoric acid in starch. *Jour. Amer. Chem. Soc.* **38**: 472–479. 1916.
18. PFEFFER, W. Physiology of plants. Translation by A. J. EWART. Vol. I: 262. University of Oxford Press. 1900.
19. PRIESTLEY, J. H. The first sugar of photosynthesis and the rôle of cane sugar in the plant. *New Phytol.* **23**: 255–265. 1924.
20. ———, and ARMSTEAD, DOROTHY. Physiological studies in plant anatomy. II. The physiological relation of the surrounding tissue to the xylem and its contents. *New Phytol.* **21**: 62–80. 1922.
21. ———, and WORMALL, A. On the solutes exuded by root pressure from vines. *New Phytol.* **24**: 24–38. 1925.
22. SACHS, J. On the physiology of plants. Translation by H. M. WARD. Page 274. University of Oxford Press. 1887.
23. SCHROEDER, J. Die Frühjahrsperiode der Birke (*Betula alba* L.) und des Ahorn (*Acer platanoides* L.). *Landw. Versuchsst.* **14**: 118–146. 1871.
24. STRASBURGER, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. *Histologische Beiträge* **3**: 591–595, 877–928. 1891.
25. WILLAMAN, J. J., and DAVISON, F. R. Some modifications of the picric acid method for sugars. *Jour. Agr. Res.* **28**: 479–488. 1924.

PHYSICAL MEASUREMENTS OF THE WINTER WHEAT PLANT AT VARIOUS STAGES IN ITS DEVELOPMENT

GEORGE JANSSEN

(WITH FOUR FIGURES)

Introduction

Winter hardiness of the wheat plant has in the main been associated with the hereditary characters of the plant. This is evidenced by the results of comparative winter wheat variety tests conducted at various experiment stations. It has been noted that modifications of this winter hardiness factor can be brought about by subjecting the same plant to different environmental conditions. This is evidenced by the work of CHANDLER (1), ROSA (18), SALMON (20), and NEWTON (14). In view of these results it might be expected that plants of the same variety of wheat, but having attained different stages of seedling development would respond differently to freezing temperatures. With this in mind a study was made of: (1) the effect of rate of thawing of frozen wheat plants at various stages of seedling growth; (2) the variations in sap concentration of winter wheat plants that had reached various degrees of development, as a result of periodical fall seedings and, (3) the relationship between the sap concentration as measured by freezing point depression, actual chemical composition, and winter hardiness.

Method

This study was conducted on a pure line of Turkey wheat, Wisconsin Pedigreed no 2. The wheat plants used for chemical purposes were taken from 1/40 acre test plats supervised by Professor B. D. LEITH, on the University farm. The seedings were made on or near the following dates: August 15, September 1, September 15, October 1, and October 15. The percentages of winter killing were determined for these various dates of seeding by making actual plant counts. These percentages of winter killing for 1923-1925 are presented in fig. 1. Material for miscellaneous freezing experiments was started in earthenware pots at the same time the field seedings were made. These potted plants were stored at the end of the fall growing season in a cool part of the greenhouse until used in the winter.

CHEMICAL ANALYSIS

All methods of chemical analyses were essentially the same as given in another paper (7).

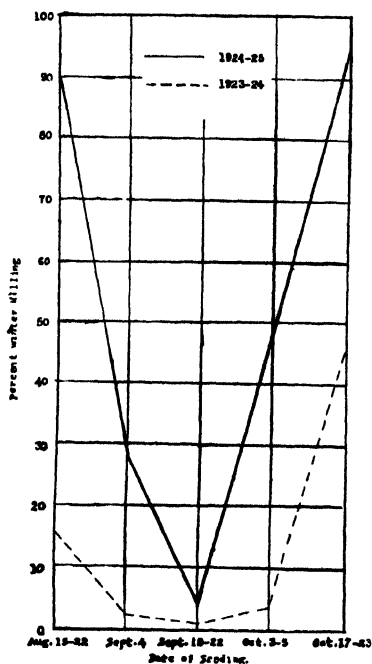


FIG. 1. Percentage of winter killing of winter wheat plants which occurred during the winter of 1923-24, and 1924-25, on five periodic seedings made during the fall season.

PHYSICAL METHODS

Samples of plants from which sap was expressed and used for physical and chemical analysis were collected and packed in jars surrounded with ice. This was done in the field when the collections were made. The samples were always kept cooled to or near 0° C. The extraction equipment was cooled in an ice bath previous to the extraction of the sap. The technique of expressing the plant sap and the determination of the freezing point was that described by GORTNER (4). As soon as collections were taken to the laboratory, the adhering soil was removed from the roots and the dead leaves cut away. The samples were then ground with an ordinary meat grinder equipped with a fine bur (peanut bur) which ground the tissue into a very fine pulp. Duplicate ten-gram samples were then taken for moisture determination. The tissue was further ground in a mortar. After grinding, 100 grams of the pulp were placed in a suitable cylinder with a two-inch bore, which was provided with a piston to which pressure could be applied by means of a hydraulic press. A pressure of 35,000 pounds was applied to the entire cylinder of tissue, which was sufficient to extract the sap, leaving the pulp in a very dry condition.

The sap was then centrifuged for five minutes at 22,000 revolutions per minute. The depression of the freezing point, total solids, and specific gravity were then determined on the centrifuged sap.

RATES OF THAWING

Much work has been done on the rate of thawing (12, 13, 19) of frozen plant tissues in an effort to determine whether or not this is a factor in winter hardiness. WEST (22) believes that at the freezing point of the plant tissue, water passes out of the cells and ice forms in the intercellular spaces. In tender fruits and vegetables when thawing occurs slowly this water will pass back into the cells if the cell walls are not ruptured. If thawing is rapid the water is not taken into the cells and the tissue dies, due to lack of water. MÜLLER-THURGAU (13) obtained injury in the pear and apple only when he employed rapid thawing. CHANDLER (1) obtained similar results with ripe apples and pears. He found also that slow thawing of lettuce was more beneficial than rapid thawing. WIEGAND (23) noted no injurious results from the rapid thawing of the frozen buds and twigs from several species of shrubs and trees. GARDNER, BRADFORD, and HOOKER (2) conclude that rapid thawing has no serious effect on frozen plant tissue.

In 1923 and 1924 the writer studied the effect of differential rates of thawing frozen wheat plants. Hardened winter wheat plants were taken from the field when the soil was frozen. At this time a recording thermograph registered the soil temperature at -5° C. in 1923 and -20° C. in 1924 at a depth of two inches below the surface. Blocks of soil two feet square and ten inches deep containing about 30 plants, were removed from the field to the greenhouse. Four samples each were taken from the plots sown on the following dates, namely, August 15, September 4, September 22, and October 5. The plants in the blocks of earth were thawed out at the following temperatures, 33° , 27° , 15° , and 5° C. Recovery data are presented in table I.

As shown in table I, of the plants frozen at -5° C., only those from the third and fourth seedings recovered in excellent condition at all four thawing temperatures. Plants from all the seedings responded equally well when thawed at 5° C. In practically all cases plants from the second, third and fourth seedings, which had been frozen at -20° C. recovered and lived when they were thawed at a temperature of 5° C., as opposed to a total loss from all seedings which occurred with higher thawing temperatures. The plants in the field from which the above samples were taken survived the winter with a minimum of killing, which seems to indicate that under outdoor conditions the gradual daily increase in temperature, noted by the daily temperature chart, allowed these plants to become adjusted to the environ-

TABLE I

GENERAL EFFECT ON THE WHEAT PLANT OF RAPID AND SLOW THAWING
PLANTS TAKEN IN FROZEN BLOCKS OF EARTH FROM THE FIELD EXPERIMENTAL PLATS, AND
THEN SUBJECTED WHILE THAWING TO THE TEMPERATURES NOTED

TEMPERATURE OF THAWING	FIRST SERIES		SECOND SERIES	
	SEEDING DATE	1923 FROZEN AT -5° C.	SEEDING DATE	1924 FROZEN AT -20° C.
		RECOVERY		RECOVERY
33° C.	Aug. 15	Very poor	Aug. 15	Killed
	Sept. 4	Fair	Sept. 4	Killed
	Sept. 22	Excellent	Sept. 22	Killed
	Oct. 5	Excellent	Oct. 5	Killed
27° C.	Aug. 15	Poor	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Killed
	Sept. 22	Excellent	Sept. 22	Killed
	Oct. 5	Fair	Oct. 5	Killed
15° C.	Aug. 15	Good	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Very poor
	Sept. 22	Excellent	Sept. 22	Very poor
	Oct. 5	Excellent	Oct. 5	3 plants lived
5° C.	Aug. 15	Excellent	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Fair
	Sept. 22	Excellent	Sept. 22	Fair
	Oct. 5	Excellent	Oct. 5	Killed

ment. This adjustment apparently was not possible for plants thawed in the greenhouse.

It is interesting, in connection with these experiments on rate of thawing, to note the effect on the plants of a second severe freeze. After the plants from the first experiment had recovered and had reached a growth condition similar to that existing before the first freezing, they were taken from their respective temperature rooms and placed under an air temperature of about -10° C. The plants taken from the room having a temperature of 5° C. were not killed by this second freezing, whereas those from higher temperatures were killed. This experiment was repeated, using plants from sowings made on different dates in 10-inch earthenware jars. These jar cultures were then stored late in the fall in a cool part of the greenhouse. The plants remained in excellent condition and were used for various experiments at intervals during the winter. In January, eight pots from each of the series sown on four dates were placed out of doors where the air temperature approximated -20° F. Two representative pots from each date series were taken into the greenhouse after 4-hour exposure and a

similar two after eight hours. These were thawed at 22° C. The remaining four pots of each date series were left outdoors until spring. The experiment was repeated one week later.

Plants which were taken into the greenhouse and thawed at a temperature of 22° C. were killed, whereas those which remained out of doors till spring recovered (see fig. 2). Immediately after the plants thawed at 22°

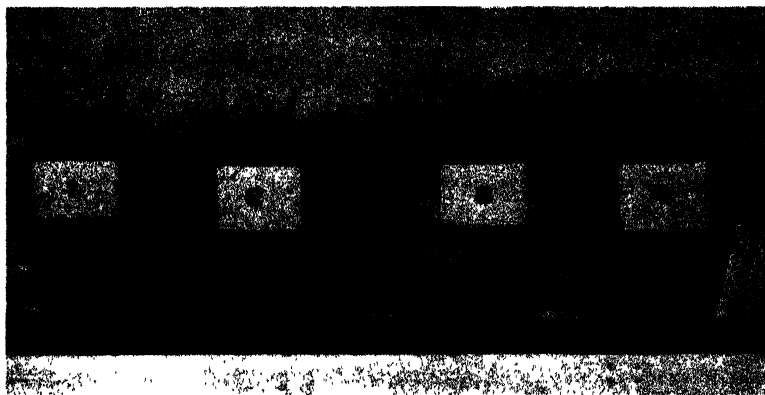


FIG. 2. Recovery of winter wheat plants from four dates of seeding (Aug. 15, Sept. 4, Sept. 22, and Oct. 5) after having been subjected to an outdoor temperature of -20° F. in January, and allowed to remain outdoors until spring. These and other similar plantings were stored in a cool part of the greenhouse where the plants remained dormant. In January, when the thermometer registered -20 F., these and other similar series of plants were placed outdoors. At the end of four and eight hour periods several series were taken into the greenhouse and allowed to thaw at a temperature of 22° C. Those plants taken into the greenhouse were all killed, while the series which remained outdoors until spring recovered in excellent condition.

C. were placed in the warm room, the leaves became flaccid and wilted. Moisture appeared on the epidermis and later evaporated, leaving the plant in a desiccated condition. The lack of available moisture in the frozen soil further tended to reduce the plant to a state of dryness from which it was not able to recover. The plants which remained out of doors were later covered with a blanket of snow, and remained in this condition until spring. The slow daily increase in outdoor temperature caused these plants to be thawed slowly. Under these conditions the plants came through the winter in excellent condition. It will be seen from fig. 2 that there is little difference in the recovery of the plants from the various dates of seeding.

A specific explanation for the recovery of the plants which remained outdoors until spring and the reason for the death of the plants which were taken into the greenhouse cannot be given. Work on protein precipitation as caused by freezing (3, 7, 21) may be suggestive. Since purified proteins

free from salts and acids are not precipitated by frost (10), we would not expect precipitation of proteins to occur in plants unless water was first removed from the tissue and the concentration of salts and acidity thereby increased. It seems that the death of these plants is caused by the loss of water, but whether the final result is directly or indirectly attributable to this condition cannot be stated. The importance of the rôle which the carbohydrate compounds play in protecting the plant against death by freezing through their ability to hold water, must not be lost sight of, and the power of the colloidal complex in the protoplasm of the plant sap and cell walls in reabsorbing the moisture which is lost during the freezing process, cannot be disregarded. Although entirely hypothetical, is it not possible to assume that in the above experiment, water was removed from the plant cells in the freezing process and that this water could have been reabsorbed by the colloidal complex if these plants had thawed slowly? This assumption takes for granted that the precipitation of the plant proteins assumed to occur at a temperature of -20° F. was reversible and that the plant would have recovered from the injurious effects of the cold, if it had been placed in an environment favorable for reversal. Such a favorable condition would account for the recovery of the plants which remained outdoors until spring. The plants which were taken into the greenhouse rapidly lost the water removed from the cells in freezing, which, with the transpiration from the leaves, left the plant in a desiccated condition, since no more water could be taken up from the frozen soil. Such a condition would tend to produce an irreversible state in the precipitated protein due to drying, which would not allow rehydration even when moisture became available.

The above is merely suggestive and certainly needs to be substantiated. The plant behavior as previously described is well established and it remains to correlate this behavior with known chemical phenomena. Chemical analyses have demonstrated that impure proteins are precipitated to a greater or lesser extent by freezing, and that carbohydrate compounds act as a protection against protein precipitation, or possibly function in the retention of water. It remains to be determined how these compounds act in the plant and how their physiological interchanges affect cold hardiness.

Physical analyses of sap expressed from plants at various stages of development

OSMOTIC PRESSURE

Recent investigations on winter hardiness have centered primarily around factors which are correlated with the ability of plants to resist low temperatures, with an attempt to obtain some specific index which might be employed in determining the hardiness of any given plant. These investi-

gations on the indices of hardiness, whether determined by physical or chemical analyses, deal largely with those factors influencing the water relationship of the plant. The consensus of opinion seems to be that any factor capable of retaining the water in the tissue of the plant, tends directly to an increased hardened condition of the plant. ROSA (18) considers the water retaining power as dependent upon the two forces, osmotic pressure and imbibition. These forces may be influenced by other factors. The correlation of the depression of the freezing point of sap with cold resistance seems to be one of the oldest means of attack upon the problems of winter hardiness. LINDLEY (11), CHANDLER (1), OHLWEILER (16), and HARRIS and POPENOE (6) obtained positive correlations between sap concentration and hardiness. PANTANELLI (17), LEWIS and TUTTLE (9), SALMON and FLEMMING (20) and NEWTON (14) concluded that the depression of the freezing point is not an index to hardiness. In a more recent publication, NEWTON (15) concludes definitely that the depression of the freezing point is not a true criterion for judging the hardiness of the plant.

A series of sap concentration measurements was made by the writer during the winter seasons of 1923, 1924, and 1925. The object of this investigation was to determine if osmotic concentration measurements as deter-

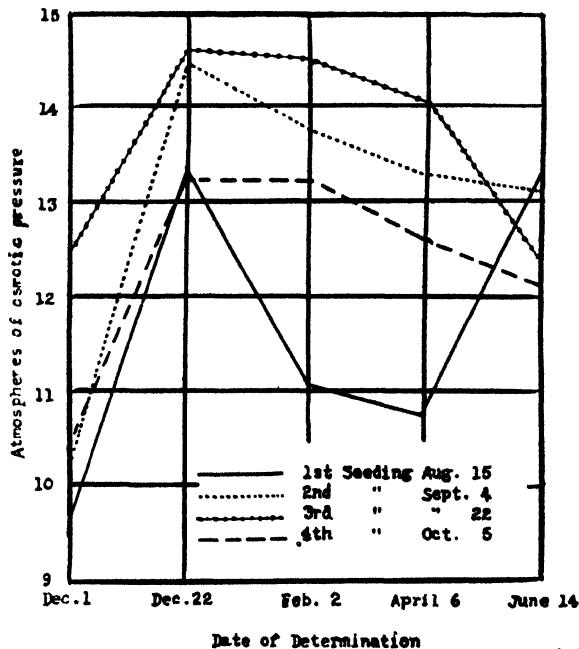


FIG. 3. Atmospheres of osmotic pressure of sap expressed from winter wheat plants at various intervals during the fall and winter months of 1923-1924. Plant collections made from four dates of seeding.

mined by the depression of the freezing point could in any way be correlated with the resistance of the winter wheat plant to cold. A series of measurements was made at intervals during the fall and winter season of 1922, 1923, and 1924, on sap extracted from plants of different dates of seeding. The data are presented in table II and fig. 3. The osmotic pressure of the sap of the wheat plant varies considerably, depending on the stage of development of the plant. In general it may be stated that for the fall and winter of 1923-1924 there was first a rise in osmotic pressure in the sap of all the plants collected up to December 22, after which it decreased gradually until the end of the dormant period, or on the termination of winter. Fig. 3 shows a distinct difference in the osmotic pressure of sap from plants grown from the seedings on different dates. Expressed sap of plants from the third date of seeding had the greatest osmotic pressure throughout the winter,

TABLE II

OSMOTIC PRESSURE, AS DETERMINED BY THE FREEZING POINT DEPRESSION OF SAP EXTRACTED FROM THE WINTER WHEAT PLANTS IN A SERIES OF ANALYSES MADE DURING THE FALL AND WINTER MONTHS OF 1923-1924
ANALYSES MADE ON PLANTS FROM FOUR DATES OF SEEDING

DATE COLLECTED	DATE OF SEEDING	OSMOTIC PRESSURE	FREEZING POINT DEPRESSION
		<i>atmospheres</i>	<i>degrees C.</i>
December 1	August 15	9.875	0.820
	September 4	10.120	0.850
	September 22	12.410	1.030
	October 4	10.360	0.860
December 22	August 15	13.400	1.125
	September 4	14.500	1.215
	September 22	14.680	1.220
	October 4	13.380	1.120
February 2	August 15	11.090	0.930
	September 4	13.840	1.150
	September 22	14.560	1.210
	October 4	13.360	1.110
April 6	August 15	10.800	0.980
	September 4	13.360	1.110
	September 22	14.080	1.170
	October 4	12.640	1.050
June 14	August 15	13.340	1.100
	September 4	13.100	1.090
	September 22	12.490	1.030
	October 4	12.160	1.020

followed closely in order by the second, first, and fourth seedings. These variations seem to be distinct, especially during the dormant period.

Results on the osmotic concentration of the wheat plant sap from plantings made in 1924 and 1925 are presented in table III. These data indicate the same general rise and fall in sap concentration as in 1923. The data, moreover, are too meager to permit conclusions for this year. In the main there is no correlation with the previous year's results. In 1923 there was

TABLE III

RANGE OF OSMOTIC PRESSURE AS DETERMINED BY DEPRESSION OF THE FREEZING POINT OF SAP EXPRESSED FROM SEEDLING WHEAT PLANTS COLLECTED FROM THE FOUR DATES OF SEEDING MADE DURING THE FALL OF 1924

DATE COLLECTED	DATE OF SEEDING			
	August 22	September 3	September 15	October 3
	<i>atmospheres</i>	<i>atmospheres</i>	<i>atmospheres</i>	<i>atmospheres</i>
September 14	8.817			
September 30	8.877			
November 6	13.840	17.80	9.25	5.844
December 8	20.440	21.52	19.24	17.320

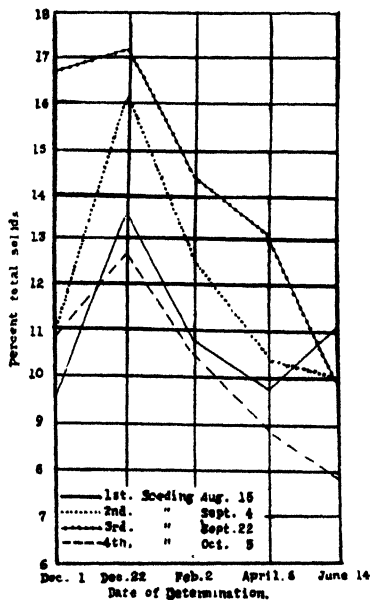


FIG. 4. Percentage of total solids in sap extracted from winter wheat plants, taken from four dates of seeding test plats at various intervals as designated. Dry matter determined with the Abbé refractometer.

a distinct correlation between sap concentration and degree of winter hardiness (see fig. 1 on winter killing).

Not in every instance were chemical analyses made on sap used in measuring the freezing point depression. No doubt such analyses would have revealed some interesting facts regarding the actual substances responsible for the depression of the freezing point. To gain some knowledge of the concentration of the sap, however, the total solids were determined. This was done with the aid of the Abbé refractometer, as described by GORTNER (5); but instead of using the sugar scale for the dry-matter reading, the total solids were obtained from Gerrling's table used in determining the total solids in sugar house products and given in the Methods of the Association of Official Agricultural Chemists. This method was checked by drying a known quantity of sap in the oven at a temperature of 70° C. A comparison of the results is given in table IV. The differences seem to fall within the range of experimental error.

TABLE IV

PERCENTAGE OF TOTAL SOLIDS IN SAP EXTRACTED FROM WINTER WHEAT PLANTS, TAKEN FROM FOUR DATES OF SEEDING ON THE TEST PLATS, AT VARIOUS INTERVALS DURING THE FALL AND WINTER OF 1923 AND 1924
RESULTS ARE GIVEN OF TOTAL SOLIDS WHEN DETERMINED WITH THE ABBÉ REFRACTOMETER, AND WHEN SAP WAS EVAPORATED IN THE OVEN AT 65°-70° C.

DATE COLLECTED	DATE OF SEEDING	REFRACTOMETER	OVEN AT 65°-70° C.
		<i>per cent.</i>	<i>per cent.</i>
December 1	August 15	9.6	9.50
	September 4	10.9	10.26
	September 22	16.8	16.70
	October 4	10.9	10.47
December 22	August 15	13.6	11.69
	September 4	16.1	14.87
	September 22	17.2	16.51
	October 4	12.7	13.28
February 2	August 15	10.8	10.197
	September 4	12.65	12.393
	September 22	14.5	14.000
	October 4	10.6	10.200
April 6	August 15	9.70	9.60
	September 4	10.45	10.00
	September 22	13.20	13.10
	October 4	8.90	8.40
June 14	August 15	11.05	11.20
	September 4	10.00	10.20
	September 22	9.80	8.10
	October 4	6.85	6.45

The data for total solids in the sap of plants from various dates-of-seeding for 1922-1923 are plotted in fig. 4. It will be noted that the total sap solids vary directly with the osmotic pressure as indicated in fig. 3. The total solids increased up to December 22, and then decreased until the opening of spring, April 6.

TABLE V

PERCENTAGE OF TOTAL SOLIDS IN SAP EXTRACTED FROM WINTER WHEAT PLANTS, TAKEN FROM FOUR DATE-OF-SEEDING TEST PLATS, AT VARIOUS INTERVALS DURING THE FALL OF 1924

DATE OF COLLECTION	DATE OF SEEDING			
	August 22	September 3	September 14	October 3
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
September 14	3.3
September 30	2.8
November 8	7.2	7.6	5.6	3.60
December 8	7.6	8.2	7.4	5.75

The 1924 results for sap solids parallel the 1924 data on osmotic pressure. There is a progressive increase in both total sap solids and osmotic pressure as the season advances. The greatest osmotic pressure was obtained on December 8 from plants of the second date-of-seeding, followed closely by the plants of the first, third, and fourth seedings. The 1924 osmotic pressure data presented in table III, however, show no correlation with similar data obtained in the fall of 1923. In the latter year, as noted previously, the sap concentration was greatest in plants from the third seeding followed in order by that in plants from the second, first, and fourth date of seeding. These discrepancies may be due to the difficulty of obtaining complete extraction from young plant tissues. Total sap solids seem to be positively correlated with the relative winter hardiness of the plants from the various dates of seeding for the year 1923, but no correlation exists for the year 1924.

Chemical composition of sap

Data on the chemical composition of sap extracted in the manner described under methods are presented in table VI. The composition of the residue is also given. The object of this experiment was to determine quantitatively the constituents of the expressed sap which was used for the determination of osmotic pressure and total solids. The analyses of the carbohydrate and nitrogenous compounds of sap extracted from plants from the

plots sown on the first date were made on October 3, 1924. It will be noted that 0.78 per cent. nitrogen, or 27.86 per cent. of the total nitrogen, was recovered in the extracted sap. Table VII gives data showing the sap composition of plants from the four dates of seeding. These data indicate that a greater proportion of both nitrogenous and carbohydrate substances are recovered in the sap of plants from the early dates of seeding. The percentage of total nitrogen in the sap ranges from 85 per cent. in plants from the first seeding, to 35.25 per cent. in plants of the fourth seeding. Similarly, the percentage of the total sugars extracted with the sap, ranges from 100 per cent. in the plants from the first seeding to 50.8 per cent. in the plants from the fourth seeding. The residue retained a greater proportion of both carbohydrate and nitrogenous compounds in young plants. Whether more of these compounds are tied up in structural materials in the younger plants, or whether the larger proportion of embryonic tissue and the smaller amount of vacuolar material in the young plant is responsible for the less complete extraction of sap in the latter, cannot be stated. The data do indicate, however, that it is the free and available substances released through grinding and extraction that determine the osmotic pressure and total solids in the plant sap, and that these readily available substances differ in character and quantity for the different dates of seeding. Thus while the data on osmotic pressure and total solids obtained in 1923 to 1924 correlate with the relative hardness of plants from the various dates of seeding for that year (see fig. 1), such correlation is not noted in data obtained for 1924 to 1925. The lack of such correlation is no doubt due to

TABLE VI

CHEMICAL COMPOSITION (CARBOHYDRATE AND NITROGEN) OF SAP EXPRESSED FROM THE CROWNS OF WINTER WHEAT PLANTS AND THE RESIDUE OF PLANTS FROM DIFFERENT DATES OF SEEDING

COMPOSITION OF SAP		COMPOSITION OF RESIDUE	PERCENTAGE REMOVED THROUGH EXTRACTION
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Total nitrogen	0.78	2.02	27.86
Protein nitrogen ..	0.35	
Filtrate nitrogen	0.43	
Total sugars	8.86	2.80	75.90
Reducing sugars . .	5.54	1.17
Sucrose	2.18	0.40
Dextrins	0.846	0.95
Starch	0.0	0.35
Hemicellulose	0.0	15.80
Soluble carbohydrate	9.706	32.84

several factors, among which may be mentioned first, a lack of correlation of hardness with the soluble carbohydrate and nitrogenous compounds in the plant at different stages of growth, and secondly the difference in degree

TABLE VII

CHEMICAL COMPOSITION (CARBOHYDRATE AND NITROGEN) OF SAP EXPRESSED FROM THE CROWNS OF WINTER WHEAT SEEDLINGS, AND OF THE RESIDUE OF PLANTS FROM DIFFERENT DATES OF SEEDING

COMPOSITION	SAP				RESIDUE			
	DATE OF SEEDING				DATE OF SEEDING			
	Aug. 22	Sept. 4	Sept. 14	Oct. 3	Aug. 22	Sept. 4	Sept. 14	Oct. 3
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Total nitrogen	1.70	1.50	1.30	1.10	0.30	1.13	1.57	2.02
Reducing sugar	9.20	6.86	4.96	5.70				
Total sugar	14.52	13.20	8.64	7.56	0.00	1.50	5.20	7.20
Dextrins					0.00	2.00	0.50	1.00
Starch					0.00	0.00	0.00	0.00
Hemicellulose					15.00	16.50	15.30	12.00

in which the plant tissue releases such soluble compounds with the ordinary methods used in the extraction of the sap. The chemical study in relation to winter killing is discussed in detail in another paper (7).

Summary

1. The percentages of winter killing of winter wheat plants, determined on date-of-seeding plats during the three years 1923 to 1925, inclusive, were found to vary with the date of seeding. The seedings were made on or close to the following dates, namely, August 15, August 31, September 21, October 6, and October 19. The average winter killing in the three years was found to be greatest in the October 19 seeding, followed in order by those of October 6, August 31, and September 21, respectively.

2. Freezing and rate-of-thawing tests on plants taken from the date-of-seeding plats show that sudden changes of temperature within a large temperature range affect the subsequent recovery of the plant. The temperature at which the plant grows, previous to freezing, is important from the standpoint of the recovery of the plant after freezing. Plants grown at high temperatures (33° C.) were killed when subjected to a freezing temperature of -10° C., whereas plants grown at a temperature of 5° C. were not seriously injured when subjected to -10° C. Rapid thawing of plants

which had been frozen at -20° C. resulted in their death. Those frozen at a temperature of -5° C. showed no serious injury when subjected to the same rapid rate of thawing.

3. In a series of osmotic pressure determinations of sap made during 1923 and 1924 on plants from the various dates of seeding, it was found that the quantity of sugars and dextrans in the sap determined the osmotic pressure; but the relative quantities of these substances taken out of the plant tissues with the extracted sap varied with the different dates of seeding. It is concluded, therefore, that the osmotic pressure measurements of sap from wheat plants at various stages of development do not indicate the degree of winter hardiness of the plants.

The writer wishes to express his appreciation to Dr. E. J. KRAUS, Dr. JAMES DICKSON, and Prof. B. D. LEITH for their helpful suggestions and criticisms given during this investigation.

DEPARTMENT OF AGRONOMY,
UNIVERSITY OF ARKANSAS.

LITERATURE CITED

1. CHANDLER, W. H. The killing of plant tissue by low temperature. Missouri Agr. Exp. Sta. Res. Bull. 8. 1913.
2. GARDNER, V. R., BRADFORD, F. C., and HOOKER, JR., H. D. Fundamentals of fruit production. McGraw-Hill Co. (See pp. 261-262.)
3. GORKE, H. Über chemische Vorgänge beim Erfrieren der Pflanzen. Landw. Vers. Stat. 65: 149-160. 1907.
4. GORTNER, R. A., and HARRIS, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. Plant World 17: 49-53. 1914.
5. ———, and HOFFMAN, W. Determination of moisture content of expressed plant tissue fluids. Bot. Gaz. 74: 308-313. 1922.
6. HARRIS, J. A., and POPENOE, W. Freezing-point lowering of the leaf sap of the horticultural types of *Persea americana*. Jour. Agr. Res. 7: 261-268. 1916.
7. JANSSEN, GEO. Effect of date of seeding of winter wheat upon some physiological changes of the plant during the winter season. Jour. Amer. Soc. Agron. 21: 168-200. 1929.
8. ———. Effect of date of seeding of winter wheat on plant development and its relationship to winter hardiness. Jour. Amer. Soc. Agron. 21: 444-466. 1929.
9. LEWIS, F. J., and TUTTLE, G. M. Osmotic properties of some plant cells at low temperatures. Ann. Bot. 34: 405-416. 1920.

10. LIDFORSS, B. Zur Physiologie und Biologie der wintergrünen Flora. *Botan. Centralbl.* **68**: 33-44. 1896.
11. LINDLEY, J. Philosophy of the destruction of plants by frost. *Trans. London Hort. Soc. Ser. 2, Vol. 2, Part IV.* Reprinted in *Horticulturist* **7**: 405-411. 1852.
12. MOLISCH, HANS. Untersuchungen über das Erfrieren der Pflanzen. 1897.
13. MÜLLER-THURGAU, H. Ueber das Gefrieren und Erfrieren der Pflanzen. *Landw. Jahrb.* **9**: 133-189. 1880.
14. NEWTON, ROBERT. A comparative study of winter wheat varieties with especial reference to winter-killing. *Jour. Agr. Sci.* **12**: 1-19. 1922.
15. ————. The nature and practical measurement of frost resistance in winter wheat. *Alberta Agr. Exp. Sta. Res. Bull.* **1**. 1924.
16. OHLWEILER, W. W. The relation between the density of cell saps and the freezing points of leaves. *Ann. Rept. Missouri Bot. Gard.* **23**: 101-131. 1912.
17. PANTANELLI, E. Sul la resistenza delle piante al freddo. *Atti. R. Acad. Lincei.* **27**: 126-130, 148-153. 1918.
18. ROSA, J. T., JR. Investigations on the hardening process in vegetable plants. *Missouri Agr. Exp. Sta. Res. Bull.* **48**. 1921.
19. SACHS, J. Krystallbildungen bei dem Gefrieren und Veränderung der Zellhäute bei dem Aufthauen saftiger Pflanzentheile. *Ber. Ver. Kön. Sachs. Gesell. Wiss. zu Leipzig* **12**: 1-50. 1860. See also *Landw. Ver. Stat.* **2**: 167-201. 1860.
20. SALMON, S. C., and FLEMING, F. L. Relation of the density of cell sap to winter hardiness in small grains. *Jour. Agr. Res.* **13**: 497-506. 1918.
21. SCHANDER, R., and SCHAFFNIT, E. Untersuchungen über das Auswintern des Getreides. *Landw. Jahrb.* **52**: 1-66. 1918.
22. WEST, F. L., and EDLIEFSEN, N. E. The freezing of fruit buds. *Utah Agr. Exp. Sta. Bull.* **151**. 1917.
23. WIEGAND, K. M. Some studies regarding the biology of buds and twigs in winter. *Bot. Gaz.* **41**: 373-424. 1906.

SOME EFFECTS OF CALCIUM DEFICIENCY ON *PISUM SATIVUM*

DOROTHY DAY

(WITH FIVE FIGURES)

Introduction

It has long been known that the addition of calcium to the soil has a beneficial effect in the growing of crops. PLINY described the chalk wells from which chalk was brought up to be spread over the surface of the soil of southern England where the land is deficient in lime. Many agricultural bulletins emphasize the need of this element, especially in growing leguminous crops.

In general, the external appearance of the plant has been employed as a standard of comparison by many investigators who have considered the effect on the plant of many variations in the conditions of growth, including the omission of certain salts from the nutrient solution.

The literature covering the necessity of calcium for the normal growth of plants and animals is vast. S'JACOB (4) finds that a deficiency of calcium produces similar results in both peas and beans. He describes the plants as somewhat limp and yellowish, with roots little branched, and often slimy by the end of the experiment. A recent investigation by CARGILL (1) shows that peas grown in a complete nutrient solution vary markedly in appearance if the ratio of calcium to magnesium is altered. In a measure her study has furnished the basis of the present investigation.

More recently workers have investigated the variations caused in the internal structure by alterations of external conditions. Before much can be accomplished in this line, it is necessary to study the structure of a normal plant, as has been done by COMPTON (2), who discusses the seedling structure of the Leguminosae. COMPTON gives a fairly clear picture of the arrangement of the principal tissues in many of the important forms, including the pea.

SOROKIN and SOMMER (8) state that the absence of calcium causes marked and rapid changes in the root tips of *Pisum sativum*, variety Golden Vine. They conclude that this element is a constituent of the protoplast essential for normal mitotic division in the meristematic region. Their evidence seems to show that a lack of calcium does not cause disintegration of tissues by separation of the cells through the absence of calcium pectate as has been suggested by some writers.

One general conception has been that calcium is important in neutralizing the acid in the soil. Doubtless this is not its only function. It has been the aim of this study to determine the effect of calcium, not with

reference to its rôle in the soil nor at the absorbing surfaces of the plant, but as a factor influencing the anatomical structure of the plant.

Materials and methods

In this investigation Canada field peas (*Pisum sativum* L.) were grown in sand cultures in the greenhouse. Fine white Ottawa quartz sand known as "general run," which RIKER (6) had previously determined to be lacking in available essential elements, was used. Earthenware jars, glazed to prevent leaching, were employed. All plants were subject to the same variations in the conditions of the greenhouse, such as light, temperature, and relative humidity.

SERIES I

In the preliminary series the composition of the nutrient solution was based upon that used by CARGILL (1) in growing peas of the same variety. Solutions of the several salts in distilled water were made up in liter quantities to serve as stock solutions. These were kept in Pyrex glass flasks tightly stoppered with corks wrapped in tinfoil. From these were compounded the three culture solutions which varied only in their content of calcium nitrate. For the complete nutrient solution (A) there was used the proportion which CARGILL (1) found to produce the greatest weight and the second greatest length for similar peas. Solution B contained one-half the amount of calcium nitrate present in solution A, while solution C lacked it entirely. In each case distilled water was added to make 1000 cc.

TABLE I
CULTURE SOLUTIONS USED IN SERIES I

SALTS	CULTURE SOLUTIONS, GRAM PER LITER		
	FULL NUTRIENT SOLUTION	SOLUTION MINUS ONE-HALF CALCIUM	SOLUTION MINUS CALCIUM
	A	B	C
	gm.	gm.	gm.
KNO ₃	0.2022	0.2022	0.2022
KH ₂ PO ₄	0.2723	0.2723	0.2723
K ₂ SO ₄	0.3485	0.3485	0.3485
FeCl ₃ · 6H ₂ O	0.0189	0.0189	0.0189
Mg(NO ₃) ₂ · 6H ₂ O	0.9115	0.9115	0.9115
Ca(NO ₃) ₂ · 4H ₂ O	0.4641	0.2321	0.0

On April 3, 1926, four seeds were placed in each of the jars containing sand moistened with the nutrient solution to be tested. It was the aim at all times to keep the sand well moistened with the respective nutrient solution, but an excess was avoided. After two weeks the two most vigorous

plants in each pot were allowed to remain while the other two were removed. Supports were inserted in the sand and the plants were tied to them. Beginning at this time and thereafter once each week, the sand at the top was flushed in the evening with distilled water which drained at the bottom through a hole uncorked for that purpose. This was repeated immediately and the jars were allowed to drain over night. The next morning the corks were replaced and enough nutrient solution was added to moisten but not to saturate the sand.

After five weeks one-third of the pots containing solution C (four plants) were discarded because of the death of the plants. The remaining pots were numbered consecutively. From this time on the complete nutrient solution (A) was added to one-half the pots (four plants). The others were treated as before. This was continued until all plants were harvested on June 14, 1926, at the end of ten weeks of growth. The pots containing solutions A and B were treated as before.

At the time of harvest observations were made as to the external aspects of stems and roots. Definite portions of stems, roots, and leaves from each plant were fixed in formalin-acetic-alcohol, embedded in parowax, sectioned, and stained. These sections were compared with similar sections of roots and stems grown in the soil under the usual garden conditions.

SERIES II

In the second series one-tenth molar solutions of the several salts in distilled water were made up in liter quantities to serve as stock solutions. Sodium nitrate was used in addition to these salts employed in series I. From these were compounded the seven culture solutions as shown in the following table. In each case distilled water was added to make 1,000 cc.

TABLE II
CULTURE SOLUTIONS USED IN SERIES II

SALTS	CULTURE SOLUTIONS, GRAM PER LITER						
	FULL NUTRI- ENT SOLUTION	SOLUTIONS MINUS ONE- HALF CALCIUM			SOLUTIONS MINUS CALCIUM		
	A	B	D	F	C	E	G
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
KNO ₃	0.2022	0.2022	0.2528	0.2780	0.2022	0.3033	0.3539
KH ₂ PO ₄	0.2723	0.2723	0.2723	0.2723	0.2723	0.2723	0.2723
K ₂ SO ₄	0.3485	0.3485	0.3485	0.3485	0.3485	0.3485	0.3485
FeCl ₃ · 6H ₂ O	0.0135	0.0135	0.0135	0.0135	0.0135	0.0135	0.0135
Mg(NO ₃) ₂ · 6H ₂ O	0.6413	0.6413	0.6413	0.6733	0.6413	0.6413	0.7054
Ca(NO ₃) ₂ · 6H ₂ O	0.2362	0.1181	0.1181	0.1181	0.0	0.0	0.0
NaNO ₃	0.0	0.0	0.4250	0.0	0.0	0.8500	0.0

In solutions A, B, and C the only difference is in the amount of calcium nitrate with the proportions of A to B to C, the same as in the earlier series, although the amount of calcium nitrate in each solution has been decreased. In solutions D and E the same ratio holds, but the nitrate content is compensated in chemically equivalent amounts by equal parts of sodium nitrate and potassium nitrate. In solutions F and G there is a similar substitution of the nitrate, but one-fourth of the nitrate was replaced by magnesium nitrate and three-fourth by potassium nitrate; therefore there is a substitution for the nitrate radical rather than a molecular substitution.

The peas were first sterilized in a weak formalin solution (1 part in 250), and rinsed in distilled water. The pots were washed in one and one-half per cent. formalin and rinsed in distilled water. On February 8, 1927, five seeds were placed in each of the jars containing sand moistened with the nutrient solution. Six jars were used for each solution. After two weeks all plants in each pot were removed except the two most vigorous ones. Supports were inserted for each plant. Beginning at this time the pots were flushed with distilled water and refilled with the nutrient solution as in the preliminary series.

At the end of five weeks' period of growth, observations were recorded as to the general appearance and height; the measurement was from the cotyledons to the top of the plant as it was growing. The plants were harvested as in the preceding series except that only one from each pot was fixed. This material was treated as in the earlier series. For the second plant green weight and dry weight determinations were made for shoots and for roots, and the material was saved for calcium analyses.

Prepared sections of roots and stems were studied microscopically and were compared as in the preceding series. In addition, the actual area of each of several tissues in every cross section of a stem or root was calculated.

Results

EXTERNAL APPEARANCE OF PLANTS

SERIES I.—A difference in the plants of the preliminary series became noticeable soon after the second week of growth and very marked by the end of five weeks. The plants starved of calcium (C) were much smaller and more chlorotic. One-third of these plants (four) had died. The twelve plants treated with solution B were taller in general than the twelve given the complete nutrient solution (A).

At the end of ten weeks the four plants starved of calcium for this entire period showed effects of the lack of this element even more than previously; in fact, about one-half of them died before this time. Those

to which the complete nutrient solution was added during the last five weeks of growth showed distinct recovery (fig. 1). Two of these elongated beyond some of the plants treated with the complete nutrient solution



FIG. 1. Plants of series I starved of calcium for the first five weeks. Pot C3 received no calcium for five subsequent weeks while pot C4 was treated with the complete nutrient solution.

throughout the entire period. It is impossible to say how far plants may be starved of calcium and yet be restored when this element is supplied.

In general, those plants given solution B were taller than those given solution A and seemed fully as vigorous. Flowers were borne on two plants only, these in solution B.

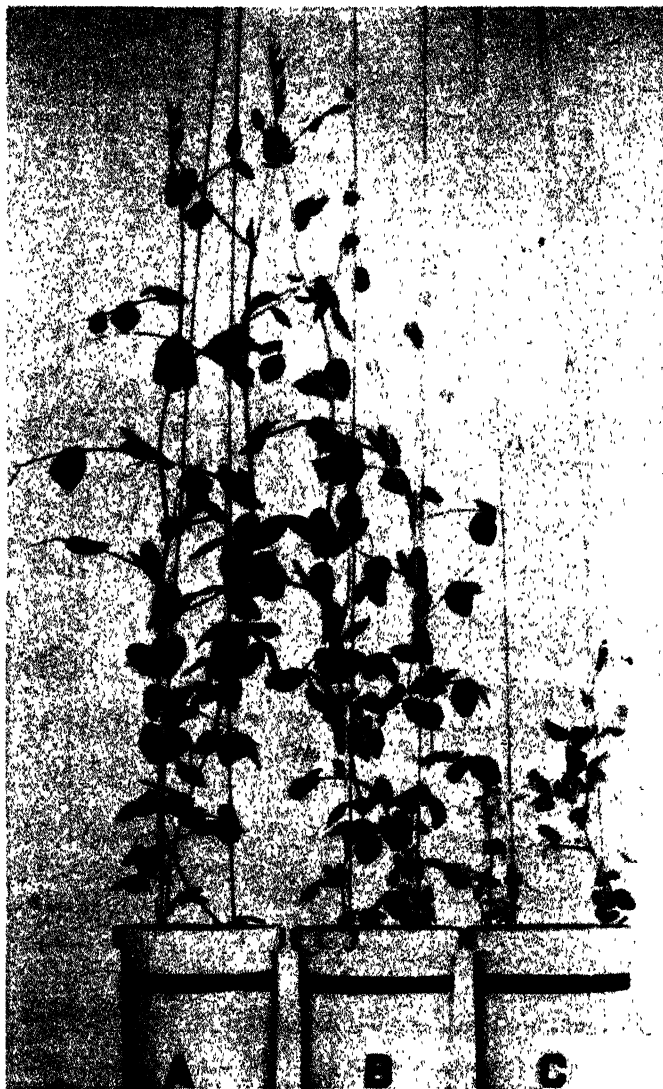


FIG. 2. Plants of series II grown in full nutrient solution (A), in a solution minus one-half the amount of calcium (B), and in a calcium-free solution (C).

SERIES II.—As in the plants of the preliminary series, a difference became noticeable soon after the second week of growth and was still more evident at the end of five weeks (fig. 2). In general, the twelve plants with the complete solution (A) seemed to be fairly uniform in height with an average of twenty-eight inches above the cotyledons. Those given the solutions with half the amount of calcium (B, D, F) showed little variation among the three groups, and were only one inch (B, D) or three inches

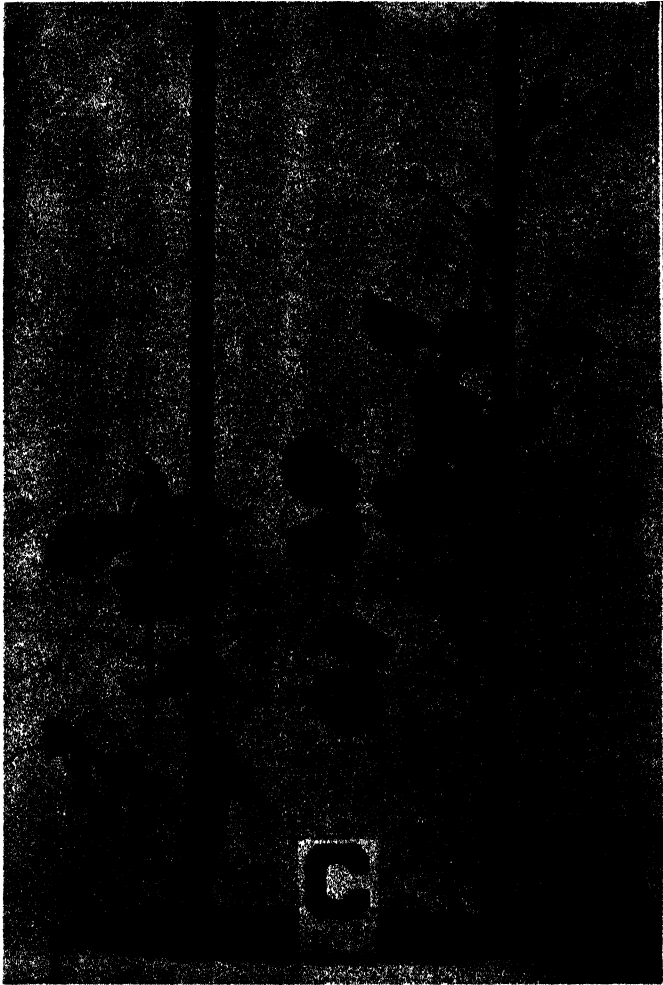


FIG. 3. Plants grown in a calcium-free solution (C). The lower leaflets and stipules show the characteristic chlorosis. In one plant the curling of the upper leaves is evident.

(F) shorter than those with the complete nutrient solution (A). There was, however, a decided difference between any of these and the plants deprived of calcium (C, E, G) although the latter showed little variation within the group as a whole. These plants were about one-half as tall as those given the complete nutrient solution (A).

The plants deprived of calcium exhibited definite changes in the leaves. The lower leaflets and stipules were chlorotic; the portions around the edge of the stipule, or leaflet, and at the base of the main vein, remained fairly green, although yellowish, while the part between these became a creamy white. The uppermost stipules and leaflets were decidedly curled and rigid almost to the point of a leathery toughness. These characteristics are best shown in a photograph of a plant treated with solution C, although all these plants were very similar (fig. 3). The plants given the half portion of calcium showed a very slight amount of curling; few or none were chlorotic.

The roots were remarkably uniform as to general appearance, being well developed with numerous secondary roots. None of them showed the short, stubby primary roots with few knobby laterals, often the case when either calcium or magnesium is in excess. No attempt was made to record the exact number or size of nodules. In general, those plants given the complete nutrient solution have more and larger nodules, those given the half amount of calcium have fewer and smaller nodules, and those deprived of calcium have no nodules, or a few extremely small ones.

ANATOMICAL STRUCTURE

Root.—In each series, sections of the primary roots cut one inch below the cotyledons showed no obvious differences in the general structure or in the proportional amounts of tissues, whether the plants were given the full amount of calcium, half that quantity, or none (fig. 4). A study of the averages (arithmetic means) given in table III shows that in the second series the plants grown in the full nutrient solution (A), when measured in cross section, were not the largest in total area, although the largest in area of the stele and area of the xylem. When these are compared in these three respects with the plants given one-half the amount of calcium (B, D, F), it is seen that the latter rank first in one case only (total area for B), but they are second from every other point of view. The plants starved of calcium (C, E, G) are the smallest in all three measurements.

If these same regions are compared to find the relation of area of stele to total area, the largest ratio is in the plants starved of calcium (E), while second are the plants given the complete nutrient solution (A). Comparisons of area of xylem to total area and of area of xylem to area

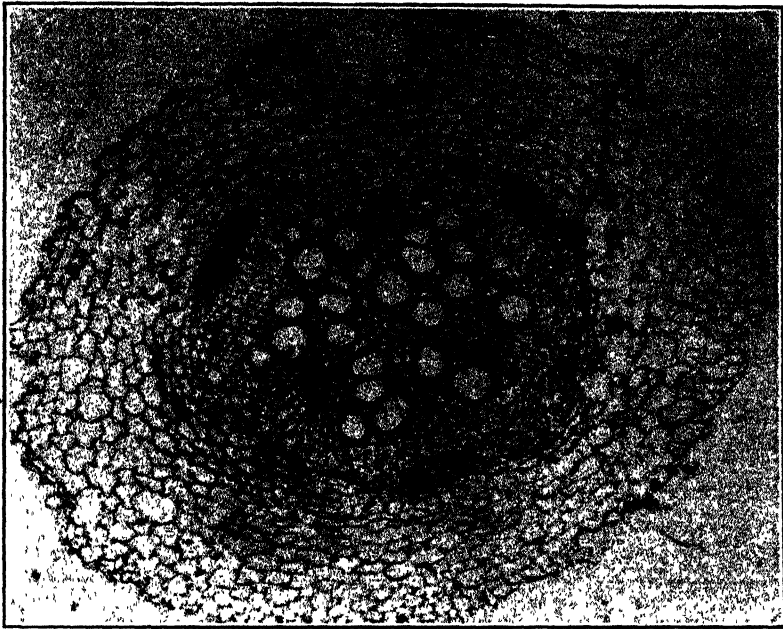


FIG. 4. Cross-section of a root cut one inch below the cotyledons of a plant starved of calcium (G). Magnification 88.

of stele show that in each case the ratio is greatest in the plants treated with the full nutrient solution (A). The plants given half the amount of calcium (B, D, F) do not show regularly a larger proportion of xylem than do those plants starved of calcium (C, E, G).

No distinct variations are noted when other sections of roots are studied in a similar but less detailed manner, although observations have been made at several levels for each root of each series. In all cases there is practically no anatomical difference between these plants and those grown under the usual garden conditions. As will appear later, the same holds true for the stem.

STEM.—In each series, sections of the main stem cut in the middle of the third internode show no striking differences as to the general structure or proportional amounts of tissues, regardless of the calcium treatment of the plant concerned (fig. 5). The averages given in table IV show that in the second series the plants in the full nutrient solution (A), when measured in cross section, are not the largest in total area, but they are largest in area of stele, and area of xylem. This was also true in the case of the root, but, for the stem, the greatest in total area is one group

TABLE III

SECTIONS OF PRIMARY ROOTS OF PEA ONE INCH BELOW THE COTYLEDONS

REGIONS MEASURED	COMPLETE NUTRIENT SOLUTION	SOLUTIONS MINUS ONE- HALF CALCIUM			SOLUTIONS MINUS CALCIUM		
	A	B	D	F	C	E	G
Total area in sq. mm.*	1.4728	1.5354	1.1476	1.1451	0.9252	0.9772	0.9425
Area of stele in sq. mm.*	0.4422	0.4145	0.3399	0.3276	0.2738	0.3009	0.2460
Area of xylem in sq. mm.*	0.2149	0.1853	0.1553	0.1478	0.1213	0.1322	0.1105
Area of stele: total area	0.3002	0.2700	0.2962	0.2861	0.2959	0.3079	0.2610
Area of xylem: total area	0.1459	0.1207	0.1353	0.1291	0.1311	0.1353	0.1172
Area of xylem: area of stele . .	0.4860	0.4470	0.4569	0.4512	0.4430	0.4393	0.4492

* Average of five plants from series II.

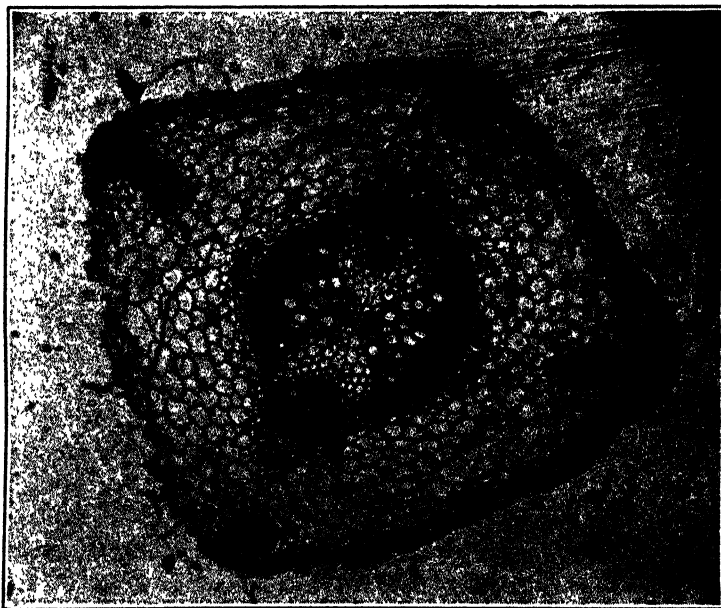


FIG. 5. Cross-section of a stem cut in the third internode of a plant starved of calcium (E). Magnification 39.

of plants starved of calcium (E), while the same plants rank second as to area of stele and area of xylem. For these three measurements no general statement can be made except that in total cross section area the plants deprived of calcium are usually larger than the plants given the half portion of calcium while the reverse is true for the area of stele and area of xylem.

If these same regions be compared to find the relation of area of stele to total area, of area of xylem to total area, and of area of xylem to area of stele, it may be seen from table IV that in each case the largest ratio is found in the plants grown in the full nutrient solution (A), while those given the half calcium (B, D, F) are second, and those starved of calcium (C, E, G) are third.

For the points just discussed, the stem and root do not follow the same variations to any marked extent.

A study of sections of the main axes at higher internodes offers no striking differences in either series. A comparison of sections taken in the fifteenth internode was attempted but the study could not be made, as some plants did not elongate to that extent. In general the number of internodes is proportional to the amount of calcium in the nutrient solution with a variation from eighteen internodes in the plants provided with the most calcium, to fourteen in those starved of calcium.

TABLE IV
SECTIONS OF STEMS OF PEA TAKEN IN THE THIRD INTERNODE

REGIONS MEASURED	COMPLETE NUTRIENT SOLUTION	SOLUTIONS MINUS ONE- HALF CALCIUM			SOLUTIONS MINUS CALCIUM		
	A	B	D	F	C	E	G
Total area in sq. mm.*	2.4915	2.2706	2.3633	2.2550	2.2777	2.5229	2.3887
Area of stele in sq. mm.*	0.6159	0.5413	0.5573	0.5369	0.4978	0.5795	0.4992
Area of xylem in sq. mm.*	0.2306	0.1934	0.1922	0.1954	0.1597	0.1981	0.1577
Area of stele: total area	0.2472	0.2384	0.2358	0.2381	0.2186	0.2297	0.2090
Area of xylem: total area	0.0926	0.0852	0.0813	0.0867	0.0701	0.0785	0.0660
Area of xylem: area of stele ...	0.3744	0.3573	0.3449	0.3639	0.3208	0.3418	0.3159

* Average of five plants from series II.

Discussion

In each series there is found the customary difference in elongation since the total length is greater and there are more internodes when calcium is present in the nutrient solution. As described by s'JACOB (4) the lower leaves of calcium starved plants "become yellow and look as if they were first sucked out by the upper leaves." Contrary to his description of the plant as limp, it has been found here that the upper leaves are stiff almost to the point of a leathery toughness. s'JACOB makes no mention of the curling of the upper leaflets and stipules. That these results seem to be due to lack of calcium is shown by those calcium-starved plants which resumed normal growth as soon as the complete nutrient solution was added.

The greater uniformity of growth and the production of some flowers by plants which received half the amount of calcium seem to indicate a better proportion of the elements in this combination than in the complete nutrient solutions. KNOP'S solution as given by PALLADIN (5) contains 0.571 gram of calcium nitrate per liter for very young plants, and 2.855 grams per liter for older plants. In SHIVE'S solution 1.228 grams of calcium nitrate per liter gave the best results with wheat and 1.842 grams per liter gave the second best (7). In this investigation the complete nutrient solution of the first series contained 0.4641 gram of calcium nitrate per liter. As compared with this amount of calcium in the nutrient solution, it is one-half this quantity that appears to give better results based on uniformity of growth of plants and on the production of flowers. Is it possible that the proportion of calcium generally used is too high?

Working with *Phaseolus vulgaris*, s'JACOB (4) decided that an excess of this element is possible, although *Pisum sativum* did not show it in his experiments. He stated that the harmful effect is due to an excess of calcium ions rather than to a high concentration of calcium carbonate. This is in disagreement with the conclusions of GILE and CARRERO (3). Whether it is purely an effect of an excess of calcium ions is not proven by this investigation, but it seems at least plausible that several nutrient solutions in common use contain more than an optimal amount of calcium.

Because the effects of the absence of calcium appeared so quickly SOROKIN and SOMMER (8) found it desirable to germinate pea seeds (*Pisum sativum*, variety Golden Vine) in a solution of calcium sulphate and then transfer the seedlings to the calcium free solutions. This variety seems to be extremely sensitive to the absence of calcium as no such difficulty was encountered in this investigation with *Pisum sativum*, variety Canada field, since all seeds were germinated in the same nutrient solution as that in which they were allowed to continue growth. It is possible that the

latter variety contains a larger amount of stored calcium thus permitting some growth at the first, followed by marked repression later in those cases where calcium was lacking.

Since their plants were grown in water cultures it is hardly possible to compare the resulting structures with those of plants grown in sand cultures. Seedlings in a nutrient solution frequently show abnormalities not evident when similar seeds are grown in pure sand treated with another portion of the same nutrient solution. However, their work agrees with the results noted by the author in the conclusion that absence of calcium does not result in separation of the cell walls. If the material examined in these studies had shown a hindrance of mitotic division such as they observed in their material it would be impossible to distinguish the tissues of normal appearance as previously noted.

In the present investigation a comparison of the anatomical structure of the root as seen in cross section showed no consistent variation whether the plant was grown under the usual garden conditions, in a complete nutrient solution, or in one which entirely lacked calcium. This holds true for sections taken at different levels. A similar comparison of stems showed no greater variation in the tissues. The only conclusion that can be reached on the basis of this investigation is that the lack of calcium causes no outstanding modification in anatomical structure. That the difference appears to be in amount of elongation, rather than in kind of structure, is supported by the measurements of the growing plants which show greater total length and have more internodes if calcium is present in the nutrient solution.

Summary

1. Canada field peas were grown in nutrient solutions in which the proportion of calcium nitrate was varied. Observations were recorded for external appearance and for anatomical structure.

2. These results support the contention that lack of calcium affects the external appearance of plants since plants starved of calcium are shorter, the lower leaves are chlorotic, and the youngest leaves are curled and tough.

3. A comparison of the proportion of calcium used here with that used by KNOP and SHIVE seems to indicate that several nutrient solutions in common use contain more than an optimal amount of this element.

4. Whether the plants are grown under the usual garden conditions, in a nutrient solution containing the full quantity of calcium, or in a solution lacking calcium, the anatomical structure of the root and of the stem remains practically constant.

5. The difference is a variation in the amount of elongation rather than in the anatomical structure as indicated by transverse sections.

This study was begun at the University of Wisconsin where most of the experimental work was completed. I wish to thank Professor J. B. OVERTON for his advice and helpful suggestions during the progress of this investigation. To Professor E. J. Kraus I desire to express my appreciation for criticism of some of the work.

DEPARTMENT OF BOTANY,
SMITH COLLEGE.

LITERATURE CITED

1. CARGILL, MIRIAM A. Antagonism between calcium and magnesium in a complete nutrient solution. Unpublished thesis, University of Wisconsin. 1925.
2. COMPTON, ROBERT HAROLD. An investigation of the seedling structure in the Leguminosae. Jour. Linn. Soc. Bot. **41**: 1-122. 1912.
3. GILE, P. L., and CARRERO, J. O. Cause of lime-induced chlorosis and availability of iron in the soil. Jour. Agr. Res. **20**: 33-62. 1920.
4. s'JACOB, J. C. Anorganische beschädigungen bij *Pisum sativum* L. en *Phaseolus vulgaris* L. Doctor's thesis, Utrecht. 1927.
5. LIVINGSTON, B. E. Palladin's plant physiology. 3d Amer. ed. pp. 82-83. Phila. 1926.
6. RIKER, R. S. Studies on the substitution of calcium, sodium, rubidium, and caesium for potassium in the growth of Hubbard squash, White Spine cucumber, and Japanese buckwheat. Unpublished thesis, University of Wisconsin. 1923.
7. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327-397. 1915.
8. SOROKIN, HELEN, and SOMMER, ANNA L. Changes in the cells and tissues of root tips induced by the absence of calcium. Amer. Jour. Bot. **16**: 23-39. 1929.

THE INSOLUBLE TYROSINASE OF THE VELVET BEAN SEED COAT

EMERSON R. MILLER

The oxidizing enzymes known as tyrosinases are widely distributed in the vegetable kingdom and are also present in invertebrates and in the skin of various animals. They play an essential rôle in the formation of black pigments in the liquids and tissues of vegetable and animal organisms.

Almost all investigations of tyrosinases so far reported concern water-soluble enzymes only. A notable exception was the discovery by GORTNER (4) that both a soluble and an insoluble tyrosinase are present in the larvae of the meal worm, *Tenebrio molitor*.

LEHMANN and SANO (7) found that *Actinomyces chromogenes* has the power of converting tyrosine into a dark brown pigment, but they could not extract any tyrosinase from the micro-organism.

The velvet bean now furnishes a very interesting and a very striking example of an insoluble enzyme which is found in the seed coat, and which has the power of oxidizing a chromogen contained only in the cotyledons, in this way producing the color, characteristic of the seeds of certain varieties.

Since this enzyme has the power of oxidizing tyrosine and other compounds containing a hydroxy-phenyl group it is classed with the tyrosinases.

Color formation in the velvet bean

While extracting powdered velvet beans with cold water it was observed that pink colored spots were soon formed throughout the mass. By diffusion, the liquid became uniformly pink and this color gradually changed through various shades of red, brown, purple and finally became black.

On examination of the mass it was found that the highly colored spots consisted mainly of fragments of seed coats. From this observation it was suspected that this formation of colored substances was due to an oxidizing enzyme, contained only in the seed coat, which was acting upon a chromogen, 3-4-dihydroxy-phenyl alanine, which occurs only in the cotyledons of the bean (8).

Several experiments were carried out in order to determine if this were the explanation of the color phenomena. Seed coats were carefully removed from several varieties of velvet beans so as to be entirely free from any interior portion of the bean. Also, cotyledons were carefully freed from any adhering seed coat. Each was then coarsely powdered.

It was found that none of the seed coats, alone, after long standing in water, produced any color. The same was true for the cotyledons of each

of the varieties examined. However, when the seed coat from any species or variety was mixed with the powdered cotyledons of that or of any other species or variety, in the presence of water, a pink color quickly appeared, usually within one minute, sometimes within half a minute or less, depending mainly upon the age of the beans and the degree of fineness of the powdered seed coats.

Action of the seed coats upon tyrosine

The seed coats from both the Early Speckled and Early White varieties produced, with a saturated aqueous solution of tyrosine, a series of colors similar to those formed when velvet bean cotyledons were used. The speed of the reaction, however, was much slower in the case of tyrosine.

Heating the dry seed coats at 105° C. for two hours diminished their activity but did not render them inactive. On the other hand, seed coats which had been mixed with water and heated in a boiling water-bath for fifteen minutes did not produce any color with velvet bean cotyledons in ten minutes. Usually, with fresh beans, color appears within half a minute.

INSOLUBILITY OF THE ENZYME

In Water.—Seed coats were triturated with clean sand and water at ordinary temperature and filtered. The turbid filtrate when mixed with velvet bean interior showed very slight activity; the *clear* filtrate showed none. The residue, however, appeared to possess the usual degree of activity.

In Other Solvents.—Fifty per cent. alcohol, various mixtures of glycerine and water, dilute salt solutions, dilute alkali and dilute acids all failed to extract anything from the seed coats that showed enzyme activity. In all these cases the residue of seed coat proved to be active.

THE INFLUENCE OF CERTAIN SUBSTANCES ON THE ACTIVITY OF THE ENZYME

A sample of coarsely powdered seed coat was allowed to stand four days in glycerine. After complete removal of the glycerine the seed coat was still very active in producing color with an aqueous extract of the velvet bean cotyledons; in the presence of fifty per cent. glycerine the activity of the enzyme was very much reduced. Color was produced in about seven minutes, while in the presence of water color appeared in one minute. This may have been due to the decrease in solubility of the chromogen and the slower rate of diffusion into the seed coat in the case of the glycerine.

Sodium chloride solutions decreased the speed of the reaction with increased concentration of the salt. With water, 1 per cent., 3 per cent., 5 per cent., 7 per cent. and 10 per cent. salt solutions, the time required to

produce noticeable change was, respectively, one minute, two minutes, two and a half minutes, four and a half minutes, ten minutes and a little more than ten minutes.

Potassium chloride produced, apparently, about the same effect as that produced by sodium chloride.

With 0.01 N mercuric chloride no color was produced in thirty minutes as against less than one minute for pure water.

With 0.01 N benzoic acid no color was observed in several hours.

With 0.2 N boric acid the speed of the reaction was reduced about fifty per cent.

Using tyrosinase from several different sources GORTNER (5) found that each of the phenols, orcin, resorcin, and phloroglucin, when added in small amount to the system, tyrosine + tyrosinase, completely prevented color formation. It has been found that these same phenols which carry two hydroxyl groups in the meta position to one another, also prevent the formation of color in a mixture of the velvet bean cotyledons and seed coats in the presence of water, or at least retard the action. In the absence of these phenols the appearance of a pink color has often been observed in less than a half minute.

INFLUENCE OF THE REACTION OF THE LIQUID ON THE ACTIVITY OF THE ENZYME

Using potato tyrosinase with tyrosine as substrate and working with buffered solutions, RAPER and WORMALL (9) found that the enzyme is most active between pH 6 and 8, and that activity ceases at pH 5 and 10.

With 3,4-dihydroxy-phenyl alanine probably no optimum pH is measurable for the velvet bean tyrosinase since very slight acidity prevents oxidation, while autooxidation occurs in even weak alkaline solutions.

Under conditions otherwise the same, 0.1 gm. of seed coat required five minutes to produce a given shade of pink color, while 0.5 gm. of seed coat required only one minute. The activity is, therefore, proportional to the mass of the seed coats used.

COMPARISON OF THE ACTIVITY OF BLACK (TRACY), MOTTLED AND WHITE SEED COATS

Using in each case the same quantity of seed coat and powdered cotyledons with a given volume of water it was found that the speed of action of the white seed coats was approximately twice as great as that of the mottled seed coats, and six to eight times that of the black seed coats. Seed coats from several samples of the black variety were used and in every case the formation of colored substances took place much more slowly than with mottled or white seed coats.

When white seed coats are mixed *in vitro* with powdered embryos of any variety of velvet bean, in the presence of water, they become uniformly dark colored.

Since the end product of the action of the oxidizing enzyme accumulates in the seed coat as the bean matures it might be expected that the more highly colored seed coats would show less activity than would be possessed by the mottled or white ones.

By cutting up mottled seed coats so as to separate the white portions from the highly colored portions it was found that the former brought about color formation more quickly than the latter. This shows that the enzyme is uniformly distributed throughout the seed coat, not in spots, and that the mottled effect is most probably due to uneven or irregular diffusion of the chromogen into the seed coat as the seed develops. In the white variety the chromogen does not seem to pass into the seed coat at all. In the black variety the chromogen evidently diffuses gradually and evenly into the seed coat, thus giving the uniform black color to the mature seed.

INFLUENCE OF AGE ON THE ACTIVITY OF VELVET BEAN SEED COATS

The cotyledons of new velvet beans are light colored, but they darken quite noticeably with age.

A sample of Early Speckled beans known to be several years old and having very dark interiors was powdered and mixed with water. Very little activity was shown; but by adding to the mixture some seed coat from a fresh sample of the same variety, considerable color formation took place in a few minutes, thus showing that it was the enzyme of the seed coat which had been affected by age.

A sample of Early White velvet beans known to be about six years old, when powdered and mixed with water, produced a pink color within five minutes. Usually, fresh beans of this variety, when so treated, show the pink color within one minute or less.

When the pink colored solution which is formed when powdered velvet beans are mixed with water is filtered from the solid material, the filtrate, on standing, passes through the same shades of color as those produced when the seed coats remain in the mixture, and also finally becomes black. Therefore, the presence of seed coats is not necessary for continuation of color formation.

The pink solution obtained by the action of MnO_2 or PbO_2 was also filtered and found to be oxidized through several shades of color, finally becoming black.

When powdered velvet beans are extracted with 95 per cent. alcohol and the solution allowed to stand in a cool place a solid separates which is almost

white. This gives the MOLISCH reaction for carbohydrate, but the carbohydrate may be removed by hot extraction with butyl alcohol, and the solid residue still gives all the reactions of 3,4-dihydroxy-phenyl alanine. When mixed with seed coats in the presence of water it shows the same color phenomena as is shown by an aqueous extract of the velvet bean cotyledons. It is evident that the chromogen extracted by means of alcohol produces the same color effects as do the aqueous extracts of the pulverized cotyledons.

Action of oxydizing enzymes from other sources

When petals of *Magnolia glauca* or *Magnolia grandiflora* are cut into strips and placed in a mixture of velvet bean cotyledons and water, a pink color is produced more quickly than by the velvet bean seed coats; and on standing, this mixture also passes through various shades of color, finally becoming black.

Aqueous solutions prepared from the petals produce the pink color even more quickly. *Magnolia* leaves also show a similar activity, but react more slowly. Also, sections of Irish potato or the filtered aqueous extract are about as active as the magnolia petals and produce practically the same color effects.

Agaricus campestris proved to be very active toward the chromogen of the velvet bean. Wheat bran, also, was quite active.

From the edible portion of the banana a solution of tyrosinase was prepared which almost instantly produced a pink color with an aqueous extract of velvet bean cotyledons. The peel of the banana was found to contain an insoluble tyrosinase which is active toward 3,4-dihydroxy-phenyl alanine.

A very active solution was also prepared from an undetermined species of mushroom having a white pileus with yellow gills.

Similar color changes are produced by some of the inorganic oxidizing agents. Thus with chemically pure manganese dioxide, or with lead dioxide, velvet bean cotyledons, in the presence of water, yield practically instantaneously a pink colored solution which passes through the usual series of colors, finally becoming black.

The pink colored solutions filtered from the residue of the dioxides, likewise, pass through the various shades of color ending in black.

It must not be supposed that the insoluble velvet bean tyrosinase is identical with the insoluble tyrosinase of the meal worm. While the enzyme of the velvet bean seed coat resembles that of the meal worm in its insolubility and in its action on tyrosine, it differs from it in having a slight oxidizing action on phloroglucinol, orcinol, pyrogallol and quinol, and by not losing its activity after standing for some time in glycerol.

Nitrogen and manganese content of the seed coats of the velvet bean

Nitrogen determinations of the seed coat of four varieties of velvet beans were made in the hope that they might throw some light on the chemical nature of the enzyme. If little or no nitrogen were present it would prove that this enzyme could not be protein-like. However, the minimum amount of nitrogen found was about 0.3 per cent. This is equivalent to about 1.87 per cent. protein, and if all the nitrogen were present in this form it would be quite possible for the enzyme to be a protein of some kind; on the other hand, it should be stated that the application of several protein tests failed to give satisfactory evidence of the presence of protein in the seed coats.

Although the nitrogen determinations did not throw any light on the chemical nature of the enzyme, yet they seem to have a bearing on the question of the deaminization of the chromogen of the velvet bean by the tyrosinase of the seed coat.

According to CHODAT and SCHWEIZER (2) tyrosinase deaminizes glycine, tyrosine and phenyl alanine. On the other hand, RAPER and WORMALL (10) did not obtain ammonia when tyrosinase acted upon tyrosine, and HAPFOLD and RAPER (6) were not able to detect the formation of ammonia when potato tyrosinase acted upon glycine, alanine or phenyl-amino-acetic acid, *unless* *p*-creosol or some other suitable phenol was present.

Judging from these results it is quite improbable that the tyrosinase of the velvet bean seed coat deaminizes 3,4-dihydroxy-phenyl alanine. In agreement with this we obtained no evidence of the formation of ammonia when velvet bean cotyledons and seed coats were mixed with water. One would naturally expect, then, that the seed coat of the black Tracy velvet bean would contain a higher percentage of nitrogen than is contained in the seed coats of the Early Speckled variety, since the latter contains much less melanin. For the same reason one would expect to find the nitrogen content of the Early White seed coats to be lower than that of the mottled varieties. On the contrary its nitrogen content is almost as high as that of the Osceola seed coat. This is not necessarily a contradiction. It was shown that the Early White seed coats showed decidedly greater enzyme activity than was shown by the seed coats of any other variety. This, of course, would mean that a larger amount of the enzyme is contained in this variety; and if the enzyme is a protein, as it may be, this would account for the higher nitrogen content of this seed coat.

The results shown in table I, based upon air dried material, were obtained by the Kjeldahl method for different samples of seed coats of the varieties indicated.

TABLE I
ANALYSES OF VELVET BEAN SEED COATS

VARIETY	NITROGEN	MOISTURE
	<i>per cent.</i>	<i>per cent.</i>
Tracy	0.662	10.09
	0.635	9.66
	0.624	
	0.626	
	0.608	
Osceola	0.343	11.58
	0.470	
	0.456	11.04
	0.463	
Early White	0.439	9.85
	0.426	
Early Speckled	0.301	10.78
	0.323	10.35
	0.335	11.14

Evidence was found that indicates the presence of more than one nitrogenous compound in the seed coat. A small quantity of seed coats of the Early Speckled variety was thoroughly extracted by each of the following solvents: water, 94 per cent. alcohol, and 0.2 per cent. aqueous solution of sodium hydroxide. The following amounts of nitrogen were extracted (Kjeldahl), based on air dried material: water, 0.014 per cent.; alcohol, 0.055 per cent.; 0.2 per cent. NaOH solution, 0.100 per cent. From these results it appears that almost all of the nitrogen of the seed coat exists in the form of an insoluble compound along with one or more other compounds present in small amount.

Analyses for manganese content of the seed coats were made because of the supposed relation of manganese to oxidase activity. In his investigations of laccase obtained from many different sources BERTRAND (1) showed that its activity was proportional to its manganese content. From *Medicago sativa* he obtained a preparation which was only slightly active and contained very little manganese. He found, however, that its activity was greatly increased by the addition of a small amount of manganese acetate or other manganese compound. From these results he concluded that manganese is an essential part of laccase.

EULER and BOLIN (3) later obtained a preparation of laccase from *Medicago sativa* which contained practically no manganese but was capable of oxidizing polyphenols in the presence of traces of manganese. Recently WIELAND and FISCHER (11) separated a plant oxidase from the juice of the fungus, *Lactarius vellereus*. Although they could detect no manganese in its ash, the enzyme was capable of oxidizing catechol and quinol, in the air, to the corresponding quinones.

From a consideration of these results and the fact that the velvet bean seed coat shows such pronounced enzymic activity, it was of interest to determine their manganese content. Table II shows amounts of manganese that were obtained by the periodate method, results being expressed in terms of the dry weight of the seed coats.

TABLE II
ANALYSES OF VELVET BEAN SEED COATS FOR MANGANESE

VARIETY	MANGANESE PER 100 GM. OF SEED COAT
	mg.
Early Speckled	23.10
Osceola	17.04
Tracy (black)	23.09
Chinese	16.15
Yokohama	16.15
Early White	23.31
Jack Bean	2.86

Although it can not be claimed that these results either confirm or disprove BERTRAND's view, yet the finding of so much manganese in a tissue having pronounced enzyme activity may be considered very suggestive. It will be noticed that the black seed coat of the Tracy variety in which there is the greatest amount of pigment, and the seed coat of the white variety which proved to be most active, are the ones which contain the greatest amounts of manganese. These facts are at least in harmony with the view that manganese is concerned in the enzyme action.

The seed coat of the Jack Bean did not, as far as it was tested, show any enzyme action, and as is shown in the table, contains much less manganese than is contained in the velvet bean seed coats.

A comparison was made of the action of velvet bean seed coats and some other substances which act as oxidizing agents, such as manganese dioxide, lead dioxide, and benzoyl peroxide, on each of the following substances in the presence of water:

(1) Dihydroxy-phenyl alanine (aqueous solution from velvet bean cotyledons); (2) orcinol; (3) phloroglucinol; (4) pyrocatechol; (5) hydroquinone; (6) benzidine; (7) pyrogallol; (8) tincture of guaiac.

The results of these comparisons are summarized in the following paragraphs:

I. Dihydroxy-phenyl alanine.

- (1) Velvet bean seed coat: pink color sometimes within twenty seconds, changing through several shades of red, brown, purple, finally becoming black.
- (2) MnO_2 : pink color practically instantaneously, quickly changing through various shades, finally black.
- (3) PbO_2 : pink color practically instantaneously, with changes similar to those in no. 1 and no. 2.
- (4) Benzoyl peroxide: a purplish pink color observed after several hours.

II. Orcinol.

- (1) Velvet bean seed coats: very slight pink after standing over night.
- (2) MnO_2 : slight red within one minute.
- (3) PbO_2 : slight color within two minutes. Reacted more quickly than the velvet bean seed coat, but more slowly than MnO_2 .
- (4) Benzoyl peroxide: very distinct pink in twelve hours.

III. Phloroglucinol.

- (1) Velvet bean seed coat: very slight yellow after standing several hours.
- (2) MnO_2 : very distinct yellowish color after standing several hours.
- (3) PbO_2 : similar to no. 2.
- (4) Benzoyl peroxide: faint yellow color within thirty minutes, but very little further change in twelve hours.

IV. Pyrocatechol.

- (1) Velvet bean seed coat: yellowish color appeared very quickly. After several hours the seed coat was almost black but the solution remained slightly pink.
- (2) MnO_2 : greenish yellow color almost immediately, finally black.
- (3) PbO_2 : yellowish color very quickly. Solution brownish after several hours.
- (4) Benzoyl peroxide: slight gray color within a few minutes; after standing over night, the liquid was yellowish brown.

V. Hydroquinone.

- (1) Velvet bean seed coat: very slight pink color within twenty minutes, changing very slowly.
- (2) MnO_2 : deep reddish brown color within fifteen minutes.
- (3) PbO_2 : slight reddish color almost immediately, but changed very slowly.
- (4) Benzoyl peroxide: very slight pink in five hours.

VI. Benzidine.

- (1) Velvet bean seed coat: after standing twenty hours the liquid had not become colored, but the seed coats had taken on a slight purple color.
- (2) MnO_2 : slight pink very quickly.
- (3) PbO_2 : the liquid was colored pink very quickly.
- (4) Benzoyl peroxide: bluish green color formed almost immediately. Became purple in a few minutes.

VII. Pyrogallol.

- (1) Velvet bean seed coat: the solution was colored yellowish within two minutes; dark brown after twelve hours.
- (2) MnO_2 : brownish red solution almost immediately, black in twelve hours.
- (3) PbO_2 : after standing several hours there was somewhat more color than was produced by velvet bean seed coat.
- (4) Benzoyl peroxide: no appreciable change within five minutes. Slight brown after long standing.

VIII. Tincture of guaiac.

- (1) Velvet bean seed coat: some samples produced a very faint greenish color in a comparatively short time, others appeared to have no effect.
- (2) MnO_2 : a pale green produced quickly.
- (3) PbO_2 : result similar to that produced by MnO_2 .
- (4) Benzoyl peroxide: also produced a pale green very quickly.

On carefully examining the color reactions described here it is seen that the behavior of the velvet bean seed coat is in general similar in its effects to that of the inorganic dioxides and benzoyl peroxide. This similarity suggests the possibility that the enzyme of the velvet bean seed coat may itself be a peroxide.

Credit is due Mr. E. F. WILLIAMS for assistance in carrying out this investigation.

LITERATURE CITED

1. BERTRAND, G. Sur l'action oxydante des sels manganoux et sur la constitution chimique des oxydases. *Compt. Rend. Acad. Sci. Paris* **124**: 1355-1358. 1897.
2. CHODAT, R., and SCHWEIZER, K. Über die deamidierende Wirkung der Tyrosinase. *Biochem. Zeitschr.* **57**: 430-436. 1913.
3. EULER, H., and BOLIN, I. Zur Kenntnis biologisch wichtiger Oxydationen. *Zeitschr. physiol. Chem.* **57**: 80-98. 1908.
4. GORTNER, R. A. A contribution to the study of the oxydases. *Jour. Chem. Soc.* **97**: 110-120. 1910.
5. ———. Studies on melanin III. The inhibitory action of certain phenolic substances upon tyrosinase. *Jour. Chem.* **10**: 113-122. 1911.
6. HAPPOLD, F. C., and RAPER, H. S. The supposed deaminising action of tyrosinase on amino acids. *Biochem. Jour.* **19**: 92-100. 1925.
7. LEHMANN, K. B., and SANO, Dr. Über das Vorkommen von Oxydationsfermenten bei Bakterien und höheren Pflanzen. *Arch. Hyg.* **67**: 99-113. 1908.
8. MILLER, EMERSON R. Dihydroxyphenylalanine, a constituent of the velvet bean. *Jour. Biol. Chem.* **44**: 481-486. 1920.
9. RAPER, H. S., and WORMALL, A. The tyrosinase-tyrosine reaction. *Biochem. Jour.* **17**: 454-469. 1923. (See also *Physiol. Rev.* **8**: 256. 1928.)
10. ———. The theory of deamination. *Biochem. Jour.* **19**: 84-91. 1925.
11. WIELAND, H., and FISCHER, F. G. Zur Frage der katalytischen Dehydrierung. *Ber. deutsch. chem. Ges.* **59**: 1180-1191. 1926.

THE DETERMINATION OF NITRATE IN GREEN TOMATO AND LETTUCE TISSUES

E. M. EMMERT

A method for the rapid and accurate determination of nitrate nitrogen in fresh plant tissues was desired in connection with some experiments on the absorption of nitrate by tomato and lettuce plants, but none seemed available. The reduction methods with Devarda alloy have been shown by RANKER (10) and others to be inaccurate. Other reduction methods seem to be subject to the same inaccuracies or are very laborious. The method in which nitric acid is reduced to nitric oxide by ferrous chloride, and the nitric oxide caught over alkali and absorbed by permanganate (9), may be accurate for fairly large amounts of nitrate, but is too laborious and requires large samples. The method suggested by EMMERT (2) in which the nitric acid is distilled from 50 per cent. by volume sulphuric acid and caught in an oxidizing solution of chlorine dioxide to reoxidize lower oxides of nitrogen, is rather laborious.

The phenoldisulphonic method seems to be the most promising method for small amounts of nitrates in soils (5). This method has been applied to plants by BURRELL and PHILLIPS (1). An alcohol extract was used, making it necessary to clear with lead acetate and destroy organic matter with sodium peroxide. Their results are very favorable, but still the process is laborious. Trials were made on water extracts by the writer. It was soon found that some agent for clarifying and stopping reduction was needed. Most gratifying results were secured by triturating green tissues with calcium hydroxide and filtering from copper hydroxide. By this means a surprisingly clear aqueous extract was secured which gave little or no interfering color with phenoldisulphonic acid. Data are presented which show that enzymes, sugars, and other reducing substances in the amounts found in tomato tissues do not reduce nitrate in solutions made strongly alkaline with calcium hydroxide.

The use of charcoal as advocated by GILBERT (4) for clearing the solution, does not seem advisable, since reduction is not stopped and extreme care has to be taken to obtain the proper form of charcoal, for occlusion and absorption cause errors, as indicated by HARPER (5).

During the writing of the present paper, a method appeared in a recent issue of PLANT PHYSIOLOGY, by HOLTZ and LARSON (6), which is somewhat similar to the method of this paper. Their method was used on dry tissue, however, and included the use of charcoal, thus subjecting itself to the dangers of GILBERT's method. Their leaching process is much longer than trituration and it does not seem as thorough in extracting nitrate as does

trituration of fresh tissue. In any drying process, changes occur no matter how carefully it is carried out. A short method of determining nitrate in green tissue enables the almost immediate analysis of tissue which has not had a chance to change to any extent. HOLTZ and LARSON may have secured complete recovery of nitrate added, yet this does not prove that all their tissue may not have suffered approximately equivalent loss of nitrate in the process of drying and during the analysis. Work with nitrate-free plant material should overcome such constant factor occurring undetected during the analysis.

It is not claimed that the method presented in this paper will give clear extracts on tissues other than those used, but if it clears these tissue extracts satisfactorily it is safe to suppose that it will be of value in other tissues.

Plants grown in normal soils do not usually contain enough chloride to interfere, so that the removal of chloride may be omitted in most cases.

Procedure

The sample is detached from the growing plant and placed in a covered container. One gram (larger amounts can be used if reagents are increased proportionately) is weighed out at once. Within fifteen minutes after weighing, the one gram sample should be placed in a mortar, 0.5 gram calcium hydroxide added, and the mixture triturated until a green (if chlorophyll is present) paste results. Care should be taken to break up all particles of tissue. Place 5 cc. of M/2 molar copper sulphate in a 50-cc. graduate and make up to exactly 50 cc. Add this to the paste in the mortar and mix thoroughly. After 5 minutes filter off a 25-cc. aliquot (a larger aliquot should be used if nitrate is low), discarding the first few cc. of filtrate. Evaporate to dryness without overheating the residue and determine nitrate by the phenoldisulphonic acid method, using sodium hydroxide as the base. It will be necessary to filter the yellow solution for reading. Addition of calcium hydroxide before filtering gives a better solution, since calcium sulphate is only slightly soluble and a precipitate of sodium sulphate does not form on standing. If more than 20 ppm. of chloride is present it must be removed by treatment with silver sulphate, as outlined by HARPER (5). Further protection against reduction may be secured by adding a cubic centimeter of toluene to the tissue at the time of trituration.

Principles involved

On trituration with calcium hydroxide the plant cells are broken up and all acidity is neutralized, free nitric acid, if present, being converted to $\text{Ca}(\text{NO}_3)_2$ before it can be reduced, which would likely take place if the

sample were triturated alone. Enzyme action probably is stimulated by trituration and, unless calcium hydroxide is present, rapid reduction of nitrate occurs (3) (7). The highly absorptive properties of copper hydroxide enable it to absorb practically all interfering substances leaving a clear solution of nitrate with traces of sugars, minerals and other substances of the plant sap. These amounts are so small that the phenoldi-sulphonic acid method for nitrates was found to give accurate results.

TABLE I

NITRATE NITROGEN FOUND IN UPPER LEAVES OF TOMATO BY TRITURATION WITH CALCIUM HYDROXIDE—PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	0.040		
2	0.0130	0.0142	0.0012
3	0.0166	0.0183	0.0017
4	0.0107	0.0142	0.0035
5	0.0081	0.0069	0.0012
6	0.0094	0.0088	0.0006
7	0.0108	0.0100	0.0008
8	0.0075	0.0075	0.0000
9	0.0085	0.0081	0.0004
10	0.0380	0.0400	0.0020
11	0.0081	0.0085	0.0004
12	0.0262	0.0238	0.0024
13	0.0250	0.0212	0.0038
14	0.0338	0.0338	0.0000
15	0.0262	0.0288	0.0026
16	0.0238	0.0175	0.0063
17	0.0276	0.0250	0.0026
18	0.0138	0.0100	0.0038
19	0.0300		
20	0.0108	0.0117	0.0009
21	0.0100	0.0133	0.0033
22	0.0092	0.0083	0.0009
23	0.0188	0.0188	0.0000
24	0.0375	0.0212	0.0163
25	0.0138	0.0175	0.0037
26	0.0092	0.0125	0.0033
27	0.0108	0.0125	0.0017
28	0.0166		
29	0.0133	0.0117	0.0016
30	0.0188	0.0163	0.0025
31	0.0150	0.0125	0.0025
32	0.0092	0.0092	0.0000
33	0.0200	0.0117	0.0083

Results

In tables I-III are presented series of nitrate determinations on tomato leaves, (I); tomato stems, (II); and lettuce leaves, (III). The soil in the plots was treated to control the reaction. It was found impossible to obtain uniform samples as to age and percentage of veins. Samples of tissue taken from the same plot might be several days or several weeks different in age, depending on their positions. Upper leaves gave 0.025 per cent. nitrogen, while lower leaves of the same plant contained 0.110 per cent. Samples taken from the same leaf might differ, since the veins contain a much larger per cent. of nitrate nitrogen and it is hard to secure the same proportion of veins in each case.

TABLE II

NITRATE NITROGEN FOUND IN TOMATO STEMS BY TRITURATION WITH CALCIUM HYDROXIDE—
PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
7	0.095	0.095	0.0
8	0.100	0.100	0.0
9	0.120	0.120	0.0
10	0.110	0.110	0.0
11	0.110	0.110	0.0
12	0.140	0.140	0.0
14	0.140	0.145	0.005
18	0.115	0.110	0.005
20	0.125	0.115	0.010
22	0.105	0.100	0.005
23	0.115	0.115	0.0
24	0.115	0.115	0.0
25	0.120	0.120	0.0
26	0.115	0.115	0.0
27	0.130	0.135	0.005
28	0.140	0.135	0.005
29	0.140	0.140	0.0
30	0.145	0.145	0.0
32	0.120	0.120	0.0

The difference between leaf and vein tissue in lettuce is shown by the fact that a sample of veins gave 0.115 per cent. nitrate nitrogen, while one from the margin of the leaves gave 0.055 per cent. Considering these points it is not justifiable to attribute the variations in tables I and III to the method. It seems more probable that they are due to differences in the samples. The fact that similar treatments were consistent in showing

TABLE III

NITRATE NITROGEN FOUND IN LETTUCE LEAVES BY TRITURATION WITH CALCIUM HYDROXIDE—
PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
1	0.250	0.237	0.013
2	0.115	0.110	0.005
3	0.085	0.080	0.005
4	0.075	0.080	0.005
5	0.090	0.100	0.010
6	0.110	0.110	0.0
7	0.113	0.113	0.0
8	0.131	0.134	0.003
9	0.125	0.131	0.006
10	0.113	0.119	0.006
11	0.125	0.100	0.025
12	0.093	0.081	0.012
13	0.100	0.115	0.015
14	0.119	0.125	0.006
15	0.113	0.119	0.006
16	0.119	0.125	0.006
17	0.100	0.113	0.013
18	0.125	0.125	0.0
19	0.125	0.134	0.009
20	0.188

like nitrate nitrogen content in most of the cases shows that the method must have a greater degree of accuracy than the data exhibit.

In table II the stems of suckers were used. In this case it was easier to secure uniform samples by using thin cross slices of stems of about the same diameter and apparent age (stems of about 0.5 cm. in diameter were used for the determinations reported in table II). The samples were handled rapidly to avoid moisture losses. Here the variation is below 5 per cent. in every case but one. This table shows that the method gives consistent results with stem tissue. The question of water loss is the big factor in variation here. As soon as the slices are cut they must be weighed at once, preferably in a covered container.

Recovery of nitrate

In order to further check the method, one gram samples of tomato leaf tissue were weighed and 5 cc. of standard nitrate solution were added to the tissue along with the calcium hydroxide. The sample was triturated and 40 cc. of water, plus 5 cc. M/2 CuSO_4 solution added. A sample of the same

TABLE IV

RECOVERY OF ADDED NITRATE NITROGEN BY TRITURATION WITH CALCIUM HYDROXIDE
ONE GRAM OF GREEN LEAVES OF TOMATO PLANTS + 0.5 MG. NITRATE NITROGEN
ADDED AS KNO_3

PLOT	N	N	N RECOVERED	DEVIATION* FROM 0.5 MG.
	<i>mg.</i>	<i>per cent.</i>	<i>mg.</i>	<i>mg.</i>
1	0.9	0.090
1 + N	1.43	..	0.53	+ 0.03
2	0.85	0.085
2 + N	1.30	0.45	- 0.05
3	0.60	0.06
3 + N	1.10	..	0.50	0.00
4	0.9	0.090
4 + N	1.4	..	0.50	0.00
5	0.58	0.058
5 + N	1.10	..	0.52	+ 0.02
6	0.60	0.06
6 + N	1.10	..	0.50	0.00
7	0.50	0.05
7 + N	1.07	..	0.57	+ 0.07
8	0.38	0.038
8 + N	0.85	..	0.47	- 0.03
9	0.55	0.055
9 + N	1.05	..	0.50	0.00
10	0.38	0.038
10 + N	0.90	..	0.52	+ 0.02

* Total + deviation = 0.14 in four cases.

Total - deviation = 0.08 in two cases.

Average deviation = 0.037.

Per cent. of error = 7.4.

TABLE V

EFFECT OF REDUCING SUGARS AND SUCROSE ON RECOVERY OF NITROGEN ADDED AS
POTASSIUM NITRATE

SAMPLE	NITROGEN ADDED	NITROGEN FOUND
	<i>mg.</i>	<i>mg.</i>
Standard solution alone	0.5	0.5
Standard solution + 3 mg. sucrose	0.5	0.5
Standard solution + 3 mg. glucose and levulose	0.5	0.5
Standard solution + 6 mg. sucrose	0.5	0.5

leaf was then analyzed in the usual way. Table IV shows the recoveries made. In four out of ten cases exact recovery was made. In only two cases was the deviation as much as 10 per cent. of the added nitrogen. In two cases the deviation was 6 per cent., and in the other two, 4 per cent. However, in only two cases was there actual loss, which would lead one to believe that the fluctuation must have been due to something besides loss of nitrate nitrogen in the procedure. The recovery of nitrate added to nitrate-free plant tissue was equally good (see table VI), the variation ranging from -10 to +16 per cent. of the added nitrate in 12 experiments, with four cases of exact recovery.

Interfering colors

In the determinations on plants containing normal amounts of nitrate no interfering colors became evident, the unknown matching the standard in excellent manner. If, however, the residue from the evaporation was overheated a faint caramel color developed, showing the presence of small amounts of organic substances. Evidently the amount of organic matter was much less than that found by BURRELL and PHILLIPS (1) using an alcohol extract. It is natural to suppose that alcoholic extracts would contain more organic matter. Alcohol also dissolves chlorophyll which is a strong reducing material, and it would naturally promote reduction of nitrate. Water, on the other hand, would not dissolve chlorophyll nor other organic substances to the degree that alcohol does. At any rate, interfering colors did not seem to be a source of error.

In the experiments reported in table VI, however, when only minute traces of nitrate were present, if any, some instances of interfering color occur, but in most cases the color was not enough to be significant. In table V interference by an amount of carbohydrate found in normal tomato tissue was not detected. A sugar solution containing 3 mg. of sugar (shown later to be the amount present in one gram of normal tomato leaves) was run through as a blank to note if any color developed when it was certain no nitrate was present. The solution after adding NaOH was perfectly colorless. The same thing was true of 6 mg., but 12 mg. of sugar gave a slight coloration, which was of a decidedly different yellow from that of the standard.

One point should be noted here, however. When ammonium hydroxide was used as the base for neutralizing the phenoldisulphonic acid, a slight color interference appeared. When sodium hydroxide was used, and the solution filtered, a solution admirably adapted for colorimetric readings was secured. On standing, a further precipitate may settle out. If calcium hydroxide is added before filtration and shaken well, calcium sulphate is

formed. Since calcium sulphate has about the same solubility, regardless of temperature, the solution remained clear on standing.

Reduction of nitrate

In strongly alkaline solution there seems to be no reduction by reducing materials present in plants. Table V indicates no loss from sugars added to nitrate solutions. A 5 per cent. solution of fresh sodium sulphate was added to a sample of standard containing 0.5 gram calcium hydroxide and the solution evaporated. Very little, if any, reduction occurred. However, when sodium hydroxide and metallic zinc were used in the presence of calcium hydroxide, the reduction was more than half.

In table V the amounts of sucrose and hydrolyzed sucrose to be added were determined by adding together the figures found by KRAUS and KRAYBILL (8) for sucrose and reducing substances in tomato leaves. Their series M was chosen, since it was the nearest to the type of soil used for the tomatoes analyzed by the writer. Since a one gram sample of tomato tissue was used for the trituration method and the highest amount of reducing substances and sucrose found by KRAUS and KRAYBILL was 0.3 per cent. of the green weight, a total of 3 mg. of sucrose were added. Reducing sugars were introduced by hydrolyzing with dilute acid. Some hydrolysis likely took place on heating the alkaline solutions of sugar and nitrate.

Table V shows that sugars had no reducing action on nitrate in the presence of calcium hydroxide. Of course, this does not prove that there are not enzymes or other substances in plants which might reduce the nitrate.

ECKERSON (3) finds that reduction in plant tissues takes place at pH 8.4 to pH 9.2, though pH 7.6 is optimum. Her figures seem to show that strong acidity checks the reducing action almost better than strong alkalinity. KASTLE and ELVOVE (7), on the other hand, found that reduction was prevented by lime-water or NaOH. Of course the amount of calcium hydroxide used in the present method would create a pH well above 10 and the excess present would maintain it thus.

In view of the contradictory findings on this point in the literature it was decided to test reduction by plant tissue still further. Although table IV gave almost complete recovery of nitrate nitrogen added, it was thought that a constant source of reduction might make it appear like complete recovery, when in reality a certain constant amount was lost in all determinations. If, however, known amounts of nitrate nitrogen were added to nitrate free tissue and complete recovery was secured, no constant error could be present. Consequently, garden bean seeds were germinated in pure white sand free of nitrate. When the cotyledon leaves were at their maximum the tissue was used. There would be no chance for nitrate nitrogen here,

TABLE VI
RECOVERY OF NITRATE ADDED TO NITRATE-FREE PLANT TISSUES

SAMPLE	N AS NITRATE ADDED	N AS NITRATE RECOVERED	NOTES ON COLOR
	<i>mg.</i>	<i>mg.</i>	
Bean	None	Faint trace of color	Seems to be faint nitrate color
"	None	Faint trace of color	Just a trace of cloudiness
"	0.1	0.100	Slight interference but reading was definite
"	0.1	0.100	No interference
"	0.2	0.188	No interference
"	0.2	0.180	Slight interference
"	0.5	0.500	No interference
"	0.5	0.500	No interference
"	1.0	0.950	No interference
"	1.0	1.050	No interference
Tomato	None	Trace of color	More color than in bean, but not significant
"	1.0	0.161	Slight interference
"	0.2	0.206	No interference
"	0.5	0.500	No interference
"	1.0	0.950	No interference

since the roots could not absorb any and it is pretty certain that all nitrogen in seeds is in the reduced form. Nitrate-free tomato tissue was secured by washing out the roots of about 8-inch plants and placing them in pure white sand. After a week considerable nitrate was still evident, as shown by the method used in the present paper, but in two weeks' time the nitrate present was negligible.

The recoveries given in table VI are rather favorable, although in some cases a little nitrogen seems to be lost. However, this is almost balanced by the cases in which too much nitrogen was found. Considering the fluctuation possible in making colorimetric readings, the writer feels that little reduction takes place in the presence of calcium hydroxide in the procedure for nitrate suggested.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF KENTUCKY.

LITERATURE CITED

1. BURRELL, R. C., and PHILLIPS, T. G. The determination of nitrate nitrogen in plants. Jour. Biol. Chem. 65: 229-234. 1925.

2. EMMERT, E. M. The chlorate method for the determination of nitrate nitrogen, total nitrogen, and other elements in soils and plant tissues. *Jour. Assoc. Offic. Agr. Chem.* **12**: 240-247. 1929.
3. ECKERSON, S. H. Protein synthesis by plants I. Nitrate reduction. *Bot. Gaz.* **77**: 377-390. 1924.
4. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates, and potassium in plant solutions. *Plant Physiol.* **1**: 191-199. 1926.
5. HARPER, H. J. The accurate determination of nitrates in soils. *Jour. Ind. Eng. Chem.* **16**: 180-183. 1924.
6. HOLTZ, H. F., and LARSON, CARL. A simple method for nitrate nitrogen determination in wheat plants. *Plant Physiol.* **4**: 288-291. 1929.
7. KASTLE, J. H., and ELVOVE, E. On the reduction of nitrates by certain plant extracts and metals, and the accelerating effect of certain substances on the progress of the reduction. *Amer. Chem. Jour.* **31**: 606-641. 1904.
8. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. *Oregon Agr. Exp. Sta. Bull.* **149**. 1918.
9. MITCHELL, H. H., SHONLE, H. A., and GRINDLEY, H. S. The origin of the nitrates in the urine. *Jour. Biol. Chem.* **24**: 461-490. 1916.
10. RANKER, E. R. Some inaccuracies of the Devarda method when applied to plant materials. *Jour. Assoc. Offic. Agr. Chem.* **10**: 252-256. 1927.

MOISTURE FLUCTUATIONS IN EXTRACTED PLANT SOLUTIONS AND IN LEAF TISSUE¹

BASIL E. GILBERT AND WALDO L. ADAMS

For a number of years workers at the Rhode Island Agricultural Experiment Station have made chemical and agronomical studies of the quantities of nutrient mineral elements necessary to insure optimum growth of field and market garden crops. Recently attempts have been made to measure the amounts of these elements found in the plant (3, 4) at various stages of growth as related to varying environmental conditions such as fertilization and weather factors. This localization of attack has involved the use of certain colorimetric chemical methods to measure small concentrations of nutrient elements found in the plant solution. Concentration has been expressed as parts per million of plant solution. Since an aqueous solution is being considered, the question arose as to the influence of environmental factors such as evaporation and soil moisture upon the concentration of the plant solution. Might there not be sufficient fluctuations in total moisture to introduce appreciable errors in the concentrations of nutrient elements in the plant solution? In order to answer this question it seemed necessary to make studies of the fluctuations occurring in the moisture content of the crop plant.

There seemed to be two fairly clear-cut ways of attacking this problem: (1) To determine the water content of the extracted plant solution at various times during the growing period. (2) To determine the total moisture content of crop plants over an entire season of growth.

Moisture content of 1926 plant solutions

In order to determine the water content of the extracted plant solution the refractometric method suggested by GORTNER and HOFFMAN (5) was used. By this method the moisture content of expressed plant tissue fluids is determined by difference, the Abbé refractometer measuring the percentage of total solids in the expressed sap in units of the sugar scale. This method is based on the fact that the refractive indices of solutions of inorganic salts and proteins in the concentrations normally present in plant sap are very nearly the same as the indices of similar solutions of carbohydrates. One or two drops of the solution expressed in the manner described by GILBERT (2) were used for each determination and the determinations were made in duplicate.

¹ Contribution no. 376 of the Rhode Island Agricultural Experiment Station.

By means of this method the average moisture percentages for the crops and tissues given in table I were determined. The crops listed were all grown on Merrimac silt loam on the Rhode Island experimental plats and in the humid climate of the coastal region.

From table I it will be noted that the standard deviation which measures the absolute variation from the mean is very small in each case. This is of interest since the determinations were made throughout the length of a growing season during which varying conditions of evaporation and soil moisture were obtained. A chart giving a description of weather observations at the Rhode Island experimental plats for the season of 1926 has already been published (4).

TABLE I

AVERAGE MOISTURE PERCENTAGES FOUND IN SOLUTIONS OF CROPS GROWN UNDER FIELD CONDITIONS IN 1926

CROP	TISSUE	NUMBER OF DETERMINATIONS	AVERAGE MOISTURE	STANDARD DEVIATION
			<i>per cent.</i>	
Parsnip	Roots	11	89.7 \pm 0.4	2.0 \pm 0.3
Beet	Roots	24	92.8 \pm 0.2	1.4 \pm 0.1
Carrot	Roots	19	93.5 \pm 0.1	0.9 \pm 0.1
Rutabaga	Roots	21	95.4 \pm 0.2	1.3 \pm 0.1
Celery	Stalks and leaves	38	95.1 \pm 0.2	1.4 \pm 0.1
Beet	Stalks and leaves	26	97.0 \pm 0.1	0.7 \pm 0.1
Buckwheat	Stalks and leaves	18	97.9 \pm 0.1	0.6 \pm 0.1
Spinach	Entire leaves	12	97.9 \pm 0.2	1.1 \pm 0.2
Lettuce	Entire leaves	21	97.5 \pm 0.2	1.1 \pm 0.1
Tomato	Branch tips	12	92.8 \pm 0.3	1.4 \pm 0.2
Potato	Branch tips	8	94.0 \pm 0.4	1.7 \pm 0.3
Corn	Tissue of stalk above uppermost node	11	96.4 \pm 0.4	2.0 \pm 0.3
Cabbage	Leaves minus midribs	47	95.1 \pm 0.1	1.3 \pm 0.1

The determinations were also made upon tissues of crops growing with both optimum and suboptimum commercial fertilization and on manured and non-manured situations. Environmental conditions, therefore, seem to have had little effect upon the concentration of the expressed plant solution. The lack of significance is also seen if the deviations are considered in the determination of an element which is present in the solution in magnitudes of 100 parts per million of solution as is the case with the nitrate ion (3).

Determinations of total moisture in 1927 and 1928

METHODS

In order to determine the total moisture, the BIDWELL-STERLING direct distillation method (1) was employed. This consists of weighing the tissue immediately after separating it from the plant, plunging it into moisture-free toluene, distilling, collecting the resulting water and reading the volume of water.

From the original weight and the volume of distilled water, the percentage of total moisture can be calculated. For plant tissue, since an appreciable error can easily be caused by exposure of cut surfaces to the air, tared weighing bottles were used. An estimated amount of tissue was introduced into the weighing bottles and tightly stoppered with a ground glass cover to prevent loss of moisture. The bottles were brought to the laboratory and weighed, and bottles and tissue were immersed in toluene. A further source of error with this method is the loss of vapor through faulty corks during distillation. This was overcome by the use of a composition cork manufactured by the Armstrong Cork Company.

In order to gain an idea as to the accuracy of this method when applied to plant tissue, several determinations were made on the same day with beet leaf blades grown in the greenhouse.

It will be seen from table II that the error of the method is small, the greatest spread being 1.5 units from the average of six determinations.

TABLE II
BIDWELL-STERLING MOISTURE DETERMINATIONS. LEAF BLADE TISSUE
OF BEETS GROWN IN THE GREENHOUSE

DETERMINATIONS	TOTAL MOISTURE
	<i>per cent.</i>
Ia	89.6
b	90.8
IIa	90.8
b	90.2
IIIa	88.6
b	88.1
Average total moisture,	
	89.6

MOISTURE CONTENT OF DIFFERENT TISSUES

In an endeavor to minimize sampling errors, comparative determinations were made on beet leaf blades and petioles; on beet leaf midribs; and on the remaining portions of the leaves after the midribs were removed.

In table III the total moisture contents as found in blades and petioles are compared. In order to evaluate the differences found, STUDENT'S method (6) has been used. By this method odds of 30:1 are usually considered significant. It will be noted that a significant difference was found when the midrib was removed from the leaf tissue.

TABLE III

PERCENTAGES OF TOTAL MOISTURE IN BEET TISSUE GROWN IN THE GREENHOUSE

LEAF BLADES	LEAF PETIOLES	MIDRIB	REMAINING PORTION
87.3	86.2	89.8	92.3
88.4	89.8	90.6	92.0
88.6	88.6	86.5	86.1
87.6	88.9	89.0	90.7
89.5	92.1	89.7	95.4
89.9	93.1	86.6	87.3
		85.8	89.4
		85.3	93.9
Average .. 88.5	89.8	87.9	90.9
Odds	16:1	Odds	79:1

Further proof of the importance of this procedure is given by the lack of significance of odds of 16:1 when leaf blades, containing midribs were compared with petioles. An average difference of 3 per cent. total moisture was found between midribs and the remaining portions of the blades. Thus, to avoid greater sampling error and considering the higher moisture content of the midrib, it has been considered wise to remove it in the case of crops where the midrib tissue is large in quantity. By this means also a tissue with less differentiation is secured and one which is more actively concerned with the processes of metabolism. This latter point is of value as in former chemical determinations upon the plant solution, an attempt has been made to select tissues which might be considered as localized areas of active metabolism.

MAXIMUM MOISTURE CHANGES

In order to assemble data concerning the loss of moisture as the plant tissue approaches the wilting condition, barley and Swiss chard were grown in sand cultures in greenhouse benches. When both crops had grown to an average height of approximately 8 inches and good leafy growth was secured, the areas were divided into equal parts. For each crop, water was supplied to one area daily while the other area was not watered. Daily de-

terminations of moisture in the leaves of barley and in the half leaves (midribs removed) of chard were made. All weighings were made immediately after severing the leaves from the plants.

In table IV the total moisture determinations on both crops are given. It will be noted that when serious wilting was observed the greatest percentage difference between the watered and unwatered plants was 7.3 per cent. with barley and 7.6 per cent. with Swiss chard. In neither case had

TABLE IV

PERCENTAGE OF TOTAL MOISTURE IN LEAF TISSUE OF CROPS GROWN IN THE GREENHOUSE

DATE	CROP	TISSUE	WATERED	UNWATERED	DIFFERENCE
Dec. 30	Barley	Entire leaf	87.4	87.3	+0.1
Jan. 11	Barley	Entire leaf	84.9	83.6	+1.3
Jan. 12	Barley	Entire leaf	86.4	83.6	+2.8
Jan. 13	Barley	Entire leaf	85.8	81.1	+4.7
Jan. 14	Barley	Entire leaf	84.1	78.6*	+5.5
Jan. 18	Barley	Entire leaf	83.6	82.9	+0.7
Jan. 20	Barley	Entire leaf	84.8	77.5*	+7.3
Jan. 16	Swiss chard	Leaves minus midrib	82.4	82.8	-0.4
Jan. 17	Swiss chard	Leaves minus midrib	89.2	88.9	+0.3
Jan. 19	Swiss chard	Leaves minus midrib	85.6	81.9	+3.7
Jan. 31	Swiss chard	Leaves minus midrib	93.9	90.0	+3.9
Feb. 1	Swiss chard	Leaves minus midrib	91.6	87.2	+4.4
Feb. 2	Swiss chard	Leaves minus midrib	89.8	86.4	+3.4
Feb. 3	Swiss chard	Leaves minus midrib	90.8	83.2*	+7.6

* Plants seriously wilted.

wilting conditions become severe enough to prevent recovery of turgor when water was supplied. Such a degree of wilting, however, is seldom seen under mesophytic field conditions such as are usual at the Rhode Island experiment station.

TOTAL MOISTURE FLUCTUATIONS IN 1928 FIELD CROPS

In order to study total moisture fluctuations under field conditions, determinations were made during the growing season of 1928 with leafy tissue of beet, tomato, celery, and corn.

With beet and corn the sampling was done so that the midrib was omitted. In sampling each of these crops, a portion of one leaf only, or a leaflet, from an individual plant was taken. The number of plants to be

sampled was determined by the relative weight of the leafy tissue and varied as follows:

	Number of plants
Tomato—leaflets near branch tips	25
Celery—leaflets near branch tips	25
Corn—cross-sections near center of lateral half of leaf-blade	50
Beet—cross-sections near center of lateral half of leaf-blade	30

These crops were grown under the same nutrient environmental variations as have already been described for the 1926 crops. Records of rainfall, soil moisture, and evaporation (by means of standard atmometers) were kept. The only correlation found was between total moisture in tissue and evaporation, there being a definite depression in the total moisture of tomato and corn leaves from July 25 to August 10, which was accompanied by conditions of quite high evaporation. However, at no time during this period was wilting observed in these crops, and the total depression in the moisture curve was no greater than 7 per cent.

In so far as was possible all tissue sampled was kept comparable with regard to size of leaf chosen, position on plants, and portion of leaf tissue. The effect of progressive maturity upon moisture content became very evident with corn. Toward the latter part of the season the percentage of moisture became materially less and as a result the standard deviation for the entire season is higher than with beet and celery (table V). This is to be expected as a very definite drying of the leaf tissue was observed visually as the season progressed.

Referring to table V it will be seen that with the four crops used in this study the standard deviations from the mean total moisture over the periods studied are very small. From this fact the conclusion may be drawn that

TABLE V

AVERAGE TOTAL MOISTURE IN CROPS GROWN UNDER FIELD CONDITIONS IN 1928

CROP	SAMPLING PERIOD	TISSUE	NUMBER OF DETERMINATIONS	TOTAL MOISTURE	STANDARD DEVIATION
	<i>weeks</i>			<i>per cent.</i>	
Beet	Sixth to twelfth	Leaves minus midribs	18	86.8 \pm 0.4	2.7 \pm 0.3
Tomato	Sixth to fifteenth	Leaflets near branch tips	32	80.8 \pm 0.5	4.1 \pm 0.3
Celery	Sixth to eleventh	Leaves minus midribs	32	78.5 \pm 0.3	2.7 \pm 0.2
Corn	Eighth to thirteenth	Leaves minus midribs	23	73.5 \pm 0.7	4.7 \pm 0.5

any error brought about in the chemical estimation of the concentration of nutrient elements in the plant solution might also be expected to be small. On a basis of 300 parts per million of nitrate ion, which is a desirable amount to maintain normal growth as shown in a former paper (3), and a maximum total moisture deviation of 5 per cent., an error of 15 parts per million would be introduced. Until the methods used in connection with studies of plant solutions are refined greatly this source of error may be disregarded when considered in connection with crops grown in normal mesophytic conditions.

Summary

Associated with studies of the quantities of certain mineral nutrient elements found in plant tissue solutions under varying conditions of nutrition and environment, data have been secured on the fluctuations of moisture in the same tissue.

1. By means of the refractometer, seasonal fluctuations in the percentage of water in the extracted tissue solution were studied.

2. The total moisture content of beet-leaf tissue has been studied and comparisons made between the content of blades, midribs, and petioles. The difference between the average moisture content of petioles and blades was found to be insignificant while midribs contained 3.0 per cent. more moisture than the remaining portion of the blade.

3. A study of the total daily moisture fluctuations in the leaf tissue of Swiss chard and barley grown under glass were made. By withholding water from a portion of each crop maximum differences of 7.6 and 7.3 per cent., respectively, in moisture content was found with both crops when wilting conditions were obtained.

4. Comparisons of soil moisture, evaporation from atmometers, and total moisture in leaf tissue were made with crops growing under field conditions. Evaporation, alone, showed evidence of correlation with moisture depression.

R. I. AGRICULTURAL EXPERIMENT STATION,
KINGSTON, R. I.

LITERATURE CITED

1. BIDWELL, G. L., and STERLING, W. F. Preliminary notes on the direct determination of moisture. *Jour. Assoc. Off. Agr. Chem.* **8**: 295-301. 1924-1925.
2. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates and potassium in plant solutions. *Plant Physiol.* **1**: 191-199. 1926.

3. ———, and HARDIN, LEO J. The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization. *Jour. Agr. Res.* **35**: 185–192. 1927.
4. ———, McLEAN, F. T. and ADAMS, W. L. The current mineral nutrient content of the plant solution as an index of metabolic limiting conditions. *Plant Physiol.* **2**: 139–151. 1927.
5. GORTNER, R. A., and HOFFMAN, W. F. Determination of moisture content of expressed plant tissue fluids. *Bot. Gaz.* **74**: 308–313. 1922.
6. "Student." The probable error of a mean. *Biometrika* **6**: 1–25. 1908.

THE EFFECT OF pH VALUE ON THE INACTIVATION TEMPERATURE OF FRUIT OXIDASE

W. Y. FONG AND W. V. CRUESS

The inactivation temperature of the oxidase of fruits is of interest in connection with the processing of fruits and fruit products by heat, as inactivation of the oxidase is essential to retention of the original fruit color.

OVERHOLSER and CRUESS (4) in 1923 reported that the organic peroxide of fresh apple juice was temporarily inactivated by 20 minutes heating at 73.5° C. and the peroxidase by 20 minutes at 90° C. While the inactivation of fruit catalase by heat was not investigated by us except incidentally, it is of interest to mention the results reported by MORGULIS, BEBER and RABKIN (3) who found that the pH value of the medium exerted a marked effect on the temperature required for inactivation of catalase from beef kidneys.

GALLAGHER (2) has reported on the behavior of the peroxidase of the mangold after heating at 100° C. but gives no data on the effect of hydrogen-ion concentration. CRUESS and FONG (1) have reported preliminary observations on the effect of pH value on the inactivation temperature of oxidase in certain fruit juices.

Procedure

In the experiments reported upon in the present paper we have attempted to determine the effect of pH value on the temperature required for complete inactivation of the peroxidase and organic peroxide of certain fruits, and the effect of pH value on the weakening action of heat on fruit peroxidase at certain temperatures below the inactivation temperature.

In one series of experiments the natural fresh juices were used; in a second series the oxidase was precipitated by alcohol and purified by redissolving in water and reprecipitation with alcohol or acetone. Usually three precipitations were used. The purified oxidase was dissolved in water and diluted to the volume of the original juice.

The natural juices and the oxidase solutions were brought to various pH values and heated at various temperatures differing by 5° C. On cooling, the heated liquids were tested for peroxidase by the addition of H_2O_2 and an oxidase indicator, usually benzidine and guaiac. Very acid or alkaline solutions, after heating, were adjusted to a pH value in which the indicators used were operative before the tests were made. For the complete oxidase, *i.e.*, peroxide and peroxidase, the indicator (usually benzidine) was added without H_2O_2 ; a positive reaction indicated the presence of organic peroxide

as well as organic peroxidase. In some experiments the approximate inactivation times of the catalase at various temperatures were also determined.

Inactivation temperature

In preliminary experiments with prune juice of pH 5.0, pear juice of pH 4.5 and peach juice of pH 3.5 it was found that the peroxidase, catalase and organic peroxide of the peach juice were inactivated at a considerably lower temperature than in the prune and pear juices. It appeared that the lower pH value of the peach juice might account for the observed difference in resistance of the oxidase and catalase to heat.

Portions of these three juices and of fresh fig juice were brought to various pH values. The approximate inactivation temperatures of the peroxidase, organic peroxide and catalase were determined and found to depend upon the pH value of the juices and not upon the variety of fruit used.

In order to test this finding further several varieties of fresh fruit juices were brought to various pH values by the addition of citric or tartaric acid, NaOH or NaHCO₃ as required. Portions of 10 cc. were heated for 10 minutes at 5° C. intervals of temperature, *e.g.* 40° C., 45° C., 50° C., etc., and the approximate inactivation temperatures determined. In other experiments the peroxidases of apricots and of peaches were precipitated with ethyl alcohol and purified by reprecipitation by alcohol from water solution. The purified peroxidases were dissolved in water, brought to various pH values, and the approximate inactivation temperatures determined as previously described.

In order to conserve space a summary only of the data will be given.

Prune¹ peroxidase in the natural juice at pH values of 2.4–2.8 was inactivated at less than 50° C.; that of pH 2.0 was inactivated at room temperature; at pH 3.1 it was inactivated at 70° C.; at pH 4.5, 90° C. was required for inactivation; at pH 5.0, 95° C. and pH 6 and 7, 100° C.

The peroxidase from green dates was somewhat more resistant. The peroxidase in tomato juice required the following temperatures for inactivation at the pH values indicated: 2.0, room temperature; pH 2.8 less than 50° C.; pH 3.0 and 3.4, 80° C.; pH 3.6, 3.8, 4.0 and 4.5, 95° C.; 6.0 and 7.0, 100° C.; and pH 8, 95° C. The peroxidase in fresh apricot juice required the following temperatures for inactivation at the pH values indicated: pH 2.6, approximately 40° C.; pH 2.8, 40–50° C.; pH 3.0 and 3.2, 60° C.; pH 3.5, 80° C.; pH 3.6, 90° C.; pH 4.0, 93° C.; pH 7.0, 98–100° C.; and at pH

¹ In this and following experiments the temperatures given are approximate as the temperature interval was 5° C. Thus an "inactivation temperature" of 50° C. signifies that the enzyme survived 45° C. but not 50° C.

10, 70–75° C. The purified peroxidase from apricots gave results similar to those given for the natural juice, although the purified oxidase was noticeably less resistant than that in the natural juice.

For the peroxidase in the natural juice expressed from ground peaches previously held in freezing storage, the following approximate inactivation temperatures were obtained: pH 2.6, 40° C. or less; pH 2.8, 50° C.; pH 3.0, 60° C.; pH 3.2, 80° C.; pH 4.0, 95° C.; pH 7.0, 100° C.; pH 8.0, 90° C.; pH 10, 85° C.; pH 12, room temperature. With the purified peroxidase the inactivation temperatures were somewhat lower, in general about 5° C. lower than for the peroxidase in the natural juice. The juices of bananas and pears behaved similarly to those of apricots and peaches.

The peaches exhibited a much stronger peroxidase reaction than did the apricots, which may account for the greater observed resistance to heat of the peroxidase in the peach juice.

The organic peroxide in the juices of peaches, apricots, pears, and prunes generally exhibited a heat resistance similar to that for the peroxidase; although in some instances probably owing to its low concentration in these cases, it was slightly less resistant to heat than the peroxidase. Whether it was destroyed by heat or whether the O_2 of the peroxide complex was merely consumed was not determined definitely, although allowing the heated juices to stand 24 hours did not result in reformation of the organic peroxide. In some cases there was evidence that, after 24–36 hours, the apparently inactivated peroxidase had regained some of its original power to produce a positive test (faint) with benzidine plus dilute H_2O_2 .

All of the juices tested at pH 2.0 were inactivated at room temperature; that is, after addition of $NaHCO_3$ to juice previously of pH 2.0, and thus bringing it to pH 7–8, tests with H_2O_2 plus benzidine and guaiac tincture were negative; similarly, with 24 hours' contact, there was found to be inactivation of the peroxidase at pH 12 at room temperature.

Our data indicate that the inactivation temperature of the peroxidase in the juices of apricots, peaches, pears, prunes, bananas, tomatoes, avocados and dates is markedly affected by the pH value of the juice. The resistance increased in general from a pH value of pH 2.0 to 7.0 and decreased from pH 8.0 to 12.0. Between pH values 3.0 and 4.5 the resistance to heat increased very rapidly, and from 4.5 to 7 slowly, or remained fairly constant. Temperatures above 100° C. were not used.

The organic peroxide behaved similarly to the peroxidase except for the fact that at pH values above 8 the organic peroxide appeared to have decomposed because of the alkalinity of the medium and failed to give positive tests even in the unheated samples.

Effect of pH value on weakening action of temperature on fruit peroxidase

Apricot juice was brought to pH values of 3.7, 5.2, 7.8 and 9.2. 100-cc. portions of juice of each pH value were heated to 50, 60, 78, 95 and 100° C. for 5 minutes. After heating and cooling, the juices were brought to approximately the same pH value. They were divided into 10-cc. portions, and a small amount of 0.3 per cent. H_2O_2 and the following peroxidase indicators were added: pyrogallol, hydroquinon, guaiacol, benzidine, p-amino-phenol, o-amino-phenol and alpha naphthol. Using the unheated samples as checks, the depth of color of the various indicators was measured comparatively after 5 hours and after 48 hours in a Klett colorimeter.

At pH 9.2 some of the indicators oxidized spontaneously even in the boiled samples. Guaiacol and benzidine were not so affected. The data for the benzidine indicator is given in table I. The untreated juice of pH 7.8 was used as the basis of comparison. As the readings of the colorimeter vary inversely with the depth of color, the reciprocals of the readings multiplied by 1,000 are given in the table. These are calculated to the basis of 100 for the untreated check of pH 7.8. The readings given were taken after 5 hours' standing; the readings taken at this time appeared to be more definite than those taken at shorter or longer intervals.

TABLE I

COLORIMETER DETERMINATION OF PEROXIDASE, WITH BENZIDINE INDICATOR

TEMPERATURE AT WHICH SAMPLE WAS HEATED	pH VALUES AND COLORIMETER READINGS			
	pH 3.7	pH 5.2	pH 7.8	pH 9.2
Room	110	140	100	91
50° C.	100	110	100	82
60° C.	91	110	100	..
78° C.	0	96	55	53
95° C.	0	10	50	53
100° C.	0	0	0	0

The results with guaiacol were similar to those reported above for benzidine, although owing to the fact that it is not so easily oxidized as benzidine the apparent inactivation temperatures were lower than those indicated for benzidine. In general the decrease in activity of the peroxidase at a given pH value with progressive increase in temperature appears to be somewhat abrupt instead of gradual. However, there is some evidence of a gradual reduction in activity. At pH 7.8, 9.2 and 3.7 the oxidase was apparently

somewhat weakened by the reaction of the medium, or the benzidine pH optimum lies near 5.2. At pH values of 5.2 and 7.8 there was also considerable indication, with hydroquinon and pyrogallol indicators, that the decrease in activity of the oxidase with increase of temperature of heating was gradual.

Summary

The temperature required for the inactivation of the peroxidase of apricots, pears, peaches, prunes, figs, lemon peel, tomato, banana and dates was found to vary with the hydrogen-ion concentration. Resistance to heat was greatest in the range of pH 5 to 7. Resistance decreased with decrease in pH value between pH 5 and 2, and decreased with increase in pH value between pH 8 and 12. At pH 12 and pH 2-2.2 the peroxidase was inactivated at room temperature in 24 hours or less.

The organic peroxide behaved similarly to the peroxidase between pH 2 and 7. At pH values above pH 8 the organic peroxide failed to give positive tests. There was considerable evidence that the inactivating effect of temperature at any given pH is progressive rather than abrupt.

FRUIT PRODUCTS LABORATORY,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. CRUESS, W. V., and FONG, W. Y. Observations on fruit oxidase. *Fruit Products Jour.* **6**: 13-15. 1926.
2. GALLAGHER, P. H. Peroxidase. Observations on the thermostability of the peroxidase of the mangold. *Biochem. Jour.* **18**: 39-46. 1924.
3. MOUGULIS, S., BEBER, M., and RABKIN, I. Studies on the effect of temperature on the catalase reaction. *Jour. Biol. Chem.* **68**: 521-533. 1926.
4. OVERHOLSER, E. L., and CRUESS, W. V. A study of the darkening of apple tissue. *California Agr. Exp. Sta. Tech. Paper* 7. 1923.



JOANNES BAPTISTA VAN HELMONT
1577-1644

BRIEF PAPERS

JOANNES BAPTISTA VAN HELMONT

(WITH ONE PLATE AND TWO FIGURES)

Plant physiology owes to the alchemist, VAN HELMONT, plate V and fig. 2, the introduction of the experimental method which he first used to determine the substances actually entering into the composition of plants. VAN HELMONT was an investigator of highest rank and the most eminent chemist of his time. His early training in medicine gave him an interest in the physiology of both plants and animals. In his study of the composition of organisms he came to the conclusion that water was the chief constituent. He arrived at this opinion both from experiments on the production of water by distillation in the analysis of organisms, and from his famous experiment on the production of plant substance by synthesis from water. The proof in his experiment rests on his belief that he had excluded all other sources of nutriment by checking the weight of soil substances absorbed. One understands quite readily, therefore, how he became convinced of the transformation of water into plant substance. However erroneous his deduction has proven to be, his experiment was well planned and his conclusion was logical on the basis of the information existing at that time on the nature of gases and the composition of the atmosphere. His son, FRANCISCUS MERCURIUS VAN HELMONT (see figures 1 and 2) has described this experiment in the collected works of the elder VAN HELMONT, which he published in 1648 under the title "*Ortus Medicinae vel Opera et Opuscula Omnia*" as follows:

"I took an earthen vessel in which I put 200 pounds of soil dried in an oven, then I moistened with rain water and pressed hard into it a shoot of willow weighing five pounds. After exactly five years the tree that had grown up weighed 169 pounds and about three ounces. But the vessel had never received anything but rain water or distilled water to moisten the soil when this was necessary, and it remained full of soil, which was still tightly packed, and, lest any dust from outside should get into the soil, it was covered with a sheet of iron coated with tin but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same 200 pounds that I started with, less about two ounces. Therefore the 164 pounds of wood, bark, and root arose from the water alone." From the translation in E. J. RUSSELL, "Soil Conditions and Plant Growth" of his "*Opera Omnia Complexionum atque Misionum Elementalium Figmentum.*"

VAN HELMONT was born of a noble Brabantine family at Brussels in 1577 and died on his estate at Vilvorde near Brussels in 1644. His life for the greater part was that of a scholar, working in quiet. His great knowledge of chemistry brought him many enticing offers from princes for his

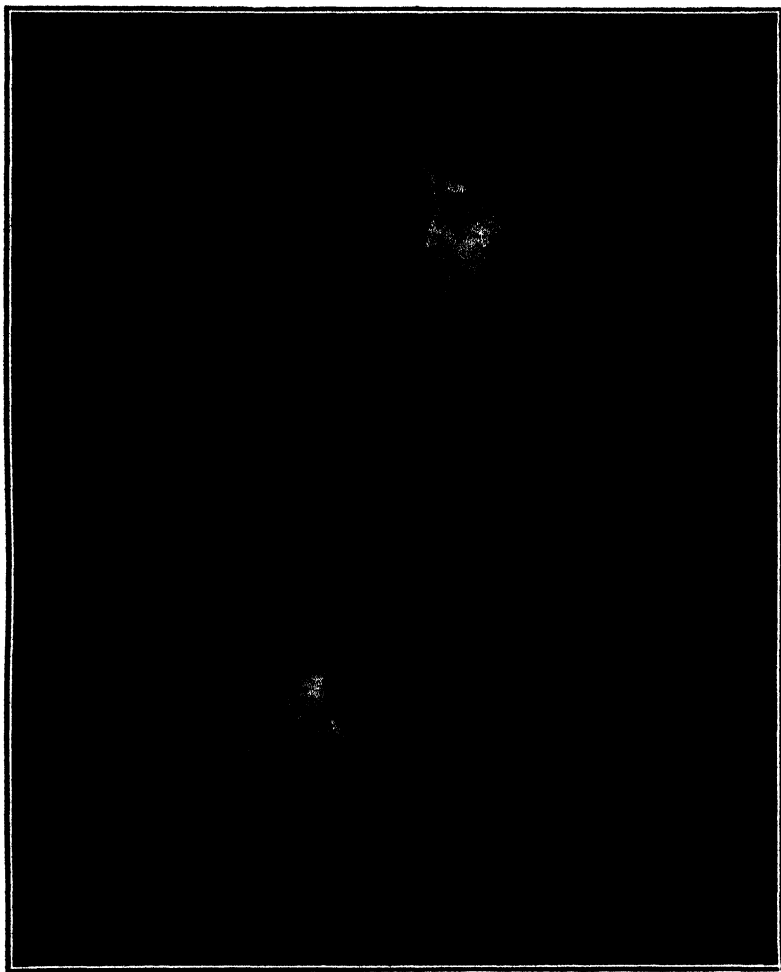


FIG. 1. FRANCISCUS MERCURIUS VAN HELMONT. From the painting by Sir Peter Lely, in the National Portrait Gallery, Trafalgar Square, London. Published by permission of the British Museum.

services, but owing to his excellent family connections and brilliant circumstances, he was able to devote without interruption his great energies to experiments in his own private laboratory. At an early age he studied

philosophy and theology and became engrossed in mysticism and the alchemistic writings. His unusually keen mind, however, soon recognized the weaknesses of the Aristotelian doctrine, that matter was composed of four elements: earth, fire, air, and water. He denied that fire could be of a material nature. It is hardly possible that he regarded air as a chemical element, for he was the first to characterize gaseous substances as differing



FIG. 2. Photograph from the memorial window in the alchemists' laboratory of the Deutsches Museum, München, Deutschland. The armorial bearings of the VAN HELMONT family from this window has been included.

in properties. He described hydrogen and methane as peculiar varieties of air. He showed that carbon dioxide could be produced from limestone or ashes by treatment with acids, from burning coal, and in the fermentation of wine and beer. He termed carbon dioxide "gas sylvestre" because he could not condense it. He introduced the term "gas," possibly having in mind the production of such substances during fermentation, the Dutch verb which means "to ferment" being "gisten."

He considered fermentation as the principal cause of digestion. He held that the acidity of the medium regulated the action of the body fluids, and showed that the acid of the gastric juice favored digestion. He compared the interactions of various digestive juices which mingle with one another with similar reactions of solutions outside of the organism, and thus laid a foundation for chemical physiology. He was a real contributor of fundamental research in a dark age of science.

A biography was published in 1922 by H. Stanley Redgrove and I. M. L. Redgrove, under the title "Joannes Baptista van Helmont."—R. B. HARVEY, *University of Minnesota*.

NOTES

Des Moines Meeting.—The program committee, under the chairmanship of Dr. W. E. LOOMIS, and with the assistance of the officers of the American Society of Plant Physiologists, is engaged in arranging the program for the Des Moines meeting. The sessions will be held on December 30 and 31, 1929, and January 1, 1930. Members are urged to attend this meeting if at all possible.

With the increase in size of the Society the Des Moines meeting should be the best yet held. A joint meeting with the Horticultural Society is to be held on Tuesday morning, December 31; and Wednesday afternoon, January 1, 1930, is reserved for a joint session with Section G of the American Association for the Advancement of Science.

Special attention is being given to the annual dinner, which has been planned for the evening of January 1, 1930, if no serious conflicts arise, and it is hoped that all members in attendance will reserve this date. Two special features of the dinner program will be the first award of the STEPHEN HALES Prize, and the fourth award of the CHARLES REID BARNES Life Membership. There will also be a brief talk by the President of the Society, and probably an illustrated address by Dr. L. G. M. BAAS-BECKING, Director of the Jacques Loeb Laboratory of the Hopkins Marine Station of Stanford University, on the work of the Laboratory.

Let us all cooperate to make the coming Des Moines meeting a live and enthusiastic affair, and a real contribution to the cause of plant physiology. There are two things that each member can do to make the meeting a great success. One is to attend the meeting; the other is to take active part in the discussion of the papers presented. The Society has already established fine traditions for its meetings, and it hopes to maintain the standards thus established.

Foreign Members.—Plant physiologists residing in foreign lands are eligible to membership in the American Society of Plant Physiologists, and our foreign members are invited to interest their friends and colleagues in such membership whenever it is possible. The dues are only slightly larger than for North American members, to cover a part of the additional postage. Those who desire membership should address Dr. H. R. KRAYBILL, Purdue University, Lafayette, Indiana.

Portrait of Van Helmont.—Again we are pleased to be able to announce that copies of the J. B. VAN HELMONT portrait are available for those who desire them for framing. The group of four, TIMIRIAZEFF,

SACHS, PFEFFER, and VAN HELMONT, may be obtained for 48 cents, postage paid. Orders may be sent to the editor of PLANT PHYSIOLOGY, with stamps or cash accompanying the order.

Manuscripts.—Members of the Society are invited to assist the Editorial Board to secure a larger volume of high class manuscripts for publication in PLANT PHYSIOLOGY. During the last year the manuscripts have been used up very closely each quarter, and with the October number we present all of the material which was available on September 15, 1929.

The Society is financially able to publish a somewhat larger volume than the current one, but cannot do so unless the manuscripts are forthcoming, and will not do so unless they are of high enough quality. Such assistance as the members may be able to render will be greatly appreciated.

Errata.—A few errors have been found in this volume of PLANT PHYSIOLOGY, and the corrections are placed at the close of the Table of Contents. It is suggested that corrections be entered at the places where the errors occur. The occasional assistance of readers in detecting such mistakes is greatly appreciated.

Fan Chi Kung.—It is with much regret that we announce the death of another member of the Society. The account of Mr. KUNG's life which is here presented has been furnished by Prof. LOUIS DeVRIES, adviser to foreign students at Iowa State College, Ames, Iowa.

Mr. FAN CHI KUNG was born on August 24, 1900, at Chengtu, Szechwan, China. Both of his parents died early and he was brought up by his grandfather, who died when he was 14 years old. He was then supported by his uncle. He entered the Junior Middle School of the Tsing Hua College, Peking, in the fall of 1915. He was graduated from the Tsing Hua College in the spring of 1923. During those eight years in Tsing Hua, he was Class President once, and held many other offices at other times. He was a member of the Executive Committee of the Tsing Hua Students' Association during the Student Movement in 1919. He came to the United States in the fall of 1923, and after staying in Cornell University for a time, he was transferred to Iowa State College, where he took up pomology, and from which institution he graduated in the summer of 1926.

Following his graduation at Iowa State College, he entered the University of Chicago, and spent a year as a postgraduate student in Plant Physiology. During the fall and winter of 1927 he was engaged in a study of the fruit industry in California, and in the spring of 1928 came back

to Iowa State College as a graduate assistant in the Pomology Section of the Iowa Agricultural Experiment Station, which position he held at the time of his death, which resulted from a car accident in the evening of July 4, 1929. He died that same night at 11:00 P.M. in the College Hospital. Burial took place in the College Cemetery.

During these few years in the United States he was Secretary of the U. S. Branch of the China Agricultural Association. He was also elected President of the Ames Cosmopolitan Club.

Physiological Basis of Drought Resistance.—In noticing the excellent work of MAXIMOW in the July number of *PLANT PHYSIOLOGY*, an unfortunate error was made in quoting the price. The book is published under the title "The plant in relation to water," is handled in this country by the Macmillan Co., and the price is \$6.50. The editor regrets the mistake. The book is worthy of a place in every physiologist's library.

Outlines of Biochemistry.—A book of unusual value to students of the biological sciences has been prepared by Dr. ROSS AIKEN GORTNER, Professor of Agricultural Biochemistry at the University of Minnesota. The "Outlines of Biochemistry" is the outgrowth of the work of the Division of Agricultural Biochemistry over a period of nearly 20 years, and represents a rich harvest of experience in handling this subject for students of the non-medical biological fields. It is particularly fortunate that the final choice of material and manner of presentation rested with Dr. GORTNER, for he is not only an active investigator with years of first hand experience with the problems of biochemistry, but is also an able and inspiring teacher, who understands the importance of clear exposition. It was the writer's privilege and pleasure several years ago to attend many of the lectures which are now summarized in this book, and a great service has been rendered in giving them permanent form.

There are seven sections to the book, the first of which, consisting of ten chapters, deals with the colloid state of matter. This section occupies almost 300 pages, and the author is particularly at home in connection with the colloidal properties of matter which are of greatest interest to biological students. The succeeding sections consider the proteins; carbohydrates and allied compounds; tannins; plant pigments; fats, lipides, and essential oils; and the biocatalysts. Following the 734 pages of text, there are general references to the literature of each section, an author index containing more than a thousand names, and an ample subject index, bringing the volume to 793 pages. Every chapter is replete with interest. The plant physiologist will be especially pleased at the amount of material

that applies more or less directly to his field. The publisher's list price is \$6.00, and orders for the book should be addressed to John Wiley and Sons, New York.

Electrostatics in Biochemistry.—With the title “Elektrostatik in der Biochemie,” a course of lectures offered at the University of Basel in October, 1928, is now issued in book form. They were originally published in the *Kolloidchemische Beihefte*, Vol. 28, Hefts 7–10. In addition to the introductory lecture and the final section of discussion, there are 18 lectures, dealing with electrostatics, electric potentials and their measurement, making of microelectrodes, pH determinations, vital staining, dispersity and particle size, dielectric constants, etc. Fourteen of the lectures are by members of the University of Prag, two from Graz, two from Basel, and one from Leipzig. It furnishes a satisfactory introduction to the field of electrostatics in biology. The publisher is Theodor Steinkopff, Dresden and Leipzig.

Physical Components of Plant Transpiration.—The second monograph from the field of scientific botany is entitled *Die physikalische Komponente der pflanzlichen Transpiration*. The author is Dr. A. SEYBOLD, of Köln, and the publisher Julius Springer, Berlin. This little monograph, dedicated to the memory of SACHS, presents the subject of transpiration in four chapters. The first deals with the physical basis of transpiration, evaporation in moving and still air, diffusion through models and porous systems, theory of psychrometers, etc. The second chapter takes up the quantitative aspects of water loss, with special reference to different types of plants. The third chapter considers the energy changes involved in the process, and the final chapter is a critical consideration of SCHIMPER's theory of restricted transpiration of xerophytic plants. A concise summary of 7 pages concludes the work. It is a good summary for those interested in the problems of water loss from plants. The price in brochure form is RM 26.

GENERAL INDEX

A

- Absorption of phosphate, 213
- Acer palmatum*, pigments, 137
- Acid hydrolysis, hemicellulose, 373
- Actions and properties of enzymes, WALD-SCHMIDT-LEITZ, 296
- Activity, fruit oxidase, effect of pH and hydrogen peroxide, 363
- ADAMS, WALDO L., Moisture fluctuations in plant solutions and tissues, 529
- Adsorption method of TSWETT, and pure pigments, 337
- Alcoholic extracts, changes in amino acids during storage, 141
- ALEXANDER, JEROME, Colloid chemistry, 163
- Alkaline hydrolysis, hemicellulose, 373
- Alkaloids, WINTERSTEIN-TRIER, 164
- Aluminum nitrate, phloridzin reagent, 357
- American Chemical Society, Division of colloid chemistry, meeting, 400
- Amino acids, changes in alcoholic extracts during storage, 141
- Analytical methods, 37, 288, 374, 412, 466, 519
- ANDERSSSEN, F. G., Seasonal changes in tracheal sap, pear and apricot, 459
- ANDREWS, F. M., Effect of temperature on flowers, 281
- WILHELM PFEFFER, 285
- Annual election, 399
- meeting, fifth, 161
- Apple, 239, 373
- starch and hemicellulose analyses, 253
- storage of hemicellulose, 251
- tissues, extraction, 239
- wood, hemicellulose estimation, 373
- Apricot, tracheal sap, seasonal changes, 459
- Arabinose, in hemicellulose, 380
- Arbutin, 413
- Atmospheric nitrogen, fixation, ERNST, 403
- Award, life membership, 161

B

- Bacteria, physiology and biochemistry, BUCHANAN and FULMER, 295

- Bartlett pear, composition of dormant shoots, 405
- Bean, velvet, insoluble tyrosinase, 507
- Beets, sugar content, testing, 367
- Benzidine, 516
- Biochemistry, electrostatics in, 549
- of bacteria, BUCHANAN and FULMER, 295
- outlines of, GORTNER, 549
- Biology, numerical data of, TERROINE and COLIN, 163
- Boron, influence on tomato, 31
- Botanical congress, fifth international, 293
- Botany, text-book, ROBBINS and RICKETT, 402
- BRODY, SAMUEL, Growth, 297
- BUCHANAN, R. E., Physiology and biochemistry of bacteria, 295
- Buffer action, in wheat sap, 224
- value, of pear and apricot sap, 460
- Burus sempervirens*, pigments, 137

C

- Calcium deficiency, effects on *Pisum sativum*, 493
- Cane, sugar, eye-spot fungus toxicity, 193
- Carbohydrates, in girasole, 125, 130
- Carotin, and TSWETT adsorption method, 337
- Cellulose, 40, 330
- Cell wall formation, mechanism, 329
- Chaenomeles lagenaria*, pigments, 136
- CHAMBERLIN, JOSEPH C., Petroleum oils as insecticides, 299
- Changes, seasonal, in sap of pear and apricot, 459
- Chemical composition of potato tuber, influence of shape, 385
- of tomato, influence of boron, 31
- reagents, extraction of pear tissues, 238
- relationship between scion and stock, citrus, 113
- Chemistry, colloid, ALEXANDER, 163
- of protoplasm, HEILBRUNN, 164
- of starch, SAMEC, 164

- SVEDBERG, 297
 of hemicellulose, 258
 CHILD, ALICE M., Shape and composition
 of potato tubers, 385
 Chlorophyll, reduction by nitrate, 202
 Chloroplast pigments, 165, 269
 seasonal variation, 135
 Chromatogram of TSWETT, 343
 Citrus species, 113, 265, 300
 identification of species colorimetrically,
 265
 CLARK-KENNEDY, A. E., Biography of
 STEPHEN HALES, 401
 Clover, red, root and shoot under long and
 short day, 438
 COLIN, H., Numerical data of biology, 163
 Colloid chemistry, ALEXANDER, 163
 Division American Chemical Society,
 meeting, 400
 of protoplasm, HEILBRUNN, 164
 of starch, SAMEC, 164
 SVEDBERG, 297
 symposium, seventh, 294
 Color interference, nitrate determination
 in green tissues, 525
 Colorimetric identification of citrus, 265
 Committee, finance, 399
 life membership, 399
 program, 399
 Components, physical, of plant transpira-
 tion, SEYBOLD, 550
 Composition, of dormant pear shoots, 405
 of potato tubers, influence of shape, 385
 of winter wheat sap, 487
 Congress, botanical, fifth international,
 293
 horticultural, international, 400
 Pacific science, fourth, 294
 Constitution, adoption, 293
 Container-size and top-root ratio, 77
 Corn, pigments and dry weight, 165
 Cosmos, root and shoot under long and
 short day, 448
 Cotton, pigment and fertilizer element,
 analyses, 273
 wilt, 208
 Council representatives, A. A. A. S., 293
 CRIST, JOHN W., Top and root size in
 herbaceous plants, 63
 Critical tables, international, 294
Crocus vernus, thermonasty, 281
 CRUESS, W. V., Fruit oxidase activity, 363
 Effect of pH value on inactivation
 temperature of fruit oxidase, 537
 CRUTCHFIELD, C. L., X-ray photography
 of mineral accumulations in plants,
 145
 Cultures, soil and solution, absorption of
 phosphate, 213
 Cytology, experimental studies, 1
- ### D
- Dahlia, root and shoot under long and
 short day, 445
 Data, numerical, of biology, TERROINE and
 COLIN, 163
 DAY, DOROTHY, Calcium deficiency of
Pisum sativum, 493
 Deficiency, calcium, effects on *Pisum sa-
 tivum*, 493
 Deflowering of tomato, 79
 Demonstration of phloridzin, 357
 Desiccation, method, 234
 Des Moines meeting, 547
 Determination, of chloroplast pigments,
 167
 of nitrate nitrogen, 288, 519
 of nitrites, 197
 Development, of root system, influence of
 day length, 435
 Dihydroxy-phenyl alanine, 515
 Distribution of nitrogen in pear sap, 249
 DORE, W. H., Influence of boron on to-
 mato, 31
 Dormant pear shoots, composition, 405
 Door of *Utricularia* trap, 87, 94
 Drought resistance, physiological basis,
 MAXIMOW, 402
 Dry weights, of tops of corn, 165
- ### E
- Election, annual, 399
 Electrostatics in biochemistry, 549
 EMMERT, E. M., Nitrate determinations in
 green tissues, 519
 Enzymes, actions and properties, WALD-
 SCHMIDT-LEITZ, 296
 ERNST, FRANK A., Fixation of atmos-

- pheric nitrogen, 403
- Errata, in vol. 4, PLANT PHYSIOLOGY, 548
- Estimation of hemicellulose, effects of acid and alkaline hydrolysis, 373
- Experimental cytology, studies in, 1
- Expression of leaf sap, critical comments, 103
- Extraction, of cytoplasmic nitrogen of pear, 240
 - of pear tissue, 233
- Extracts, alcoholic, changes during storage, 141
- Eye-spot fungus, toxicity in sugar cane, 193

F

- Factors, limiting, in use of petroleum oil insecticides, 299
- Fermentation, problem of, SCHOEN, M., translation by HIND, 163
- Ferric chloride, phloridzin reagent, 357
- Fertilizing, potassium, nitrogen, phosphorus, effects on chloroplast pigments, mineral content, and production, 269
- Fifth annual meeting, 161
 - international botanical congress, 293
- Finance committee, 399
- Fixation, atmospheric nitrogen, ERNST, 403
- Flowering, of wheat, water content of leaves at time of, 393
- Flowers, temperature effect, 281
- Fluctuations of moisture in plant solutions and tissues, 529
- FONG, W. Y., Fruit oxidase activity, 363
 - Effect of pH value on inactivation temperature of fruit oxidase, 537
- Foreign members, 547
- Forest tree seeds, 162
- Formation of cell wall, mechanism, 329
- Fourth Pacific science congress, 294
- Freezing, pear tissues, 237
- Fruit oxidase activity, pH value and, 363
 - peroxide concentration and, 363
 - inactivation temperature, 532
- Fruits, of tomato, growth and respiration studies, 349
- FULMER, E. I., Physiology and biochemistry of bacteria, 295

- Fungus, eye-spot, toxicity to sugar cane, 193

G

- Galacturonic acid, 40
- GARDNER, F. E., Temperature influence on dormant pear shoots, 405
- Genetical purposes, testing beets for, 367
- GERHARDT, FISK, Estimation of hemicellulose in apple wood, 373
- Germination, 3
- GILBERT, BASIL E., Moisture fluctuations in plant solutions and tissues, 529
- Ginkgo, pigments, 137
- Girasole, storage, 123
- Glucose, relation to cellulose, 330, 333
- GORTNER, R. A., Outlines of biochemistry, 549
- Grapefruit, 113, 265
- GREENE, CHARLES WILSON, Growth, 297
- Growth, ROBBINS, BRODY, HOGAN, JACKSON, and GREENE, 297
 - initiation, of dormant pear shoots, 405
 - of roots in plaster molds, 15
 - of roots under lateral pressure, 18
 - of tomato, influence of boron, 31, 50
 - rates, of corn, 176
 - responses, pear shoots, 415
 - studies on fruits, tomato, 349
- Guaiae, tincture, color changes, 516
- GUSTAFSON, F. G., Respiration of tomato fruits, 349

H

- HAAS, A. R. C., Chemical relationship, scion and stock, citrus, 113
 - Colorimetric identification of citrus, 265
- HALES, STEPHEN, award, 162
 - biography, CLARK-KENNEDY, 401
- HALMA, F. F., Chemical relationship, scion and stock, citrus, 113
 - Colorimetric identification of citrus, 265
- HARVEY, E. M., Microchemical demonstration of phloridzin, 357
- HARVEY, R. B., JULIUS VON SACHS, 155
- JOANNES BAPTISTA VAN HELMONT, 543
- Heat, bottom, influence on top-root relation, 79
 - effect on toxic substance, eye-spot fungus, 197

- HEILBRUNN, L. V., Colloid chemistry of protoplasm, 164
- Helianthus tuberosus*, storage, 123
- Helminthosporium sacchari*, toxic to sugar cane, 193
- Hemicellulose, 39
estimation by acid and alkaline hydrolysis, 373
storage in woody plants, 251
- Herbaceous plants, top and root size relations, 63
- HIMMEL, W. J., Root and shoot relation under long and short day, 435
- HIND, H. L., Translation of SCHOEN, M., Problem of fermentation, 163
- HOGAN, ALBERT GARLAND, Growth, 297
- HOLTZ, H. F., Nitrate nitrogen determination in wheat plants, 288
- Horse chestnut, chlorophyll, 138
- Horticultural congress, international, 400
- HOTTES, CHARLES F., Studies in experimental cytology, 1
- HURD-KARRER, ANNIE M., Water content of wheat leaves at flowering, 393
- Hydrogen ion concentration, and fruit oxidase activity, 363
and inactivation temperature of oxidase, 537
of wheat sap, 224
peroxide concentration and fruit oxidase activity, 363
- Hydrolysis, acid and alkaline, of hemicellulose, 373
effect on extraction, 247
- Hydroquinone, color changes, 516
- Hydrostatic-pneumatic system of trees, MACDOUGAL, OVERTON, and SMITH, 297
- Hydroxyl ion, influence on protein extraction from pear, 246
- I
- Identification of citrus species, colorimetric, 265
- Illumination, long and short day, effect on root and top, 435
- Inactivation temperature, fruit oxidase, 537
- Insecticides, saturated petroleum oils as, 299
- Insoluble tryosinase, in velvet bean, 507
- International congress, fifth botanical, 293
horticultural, 400
critical tables, 294
- Inulin, in girasole, 125
- Iresine herbati*, expression of sap, 108
- Iris, root and shoot under long and short day, 443.
- J
- JACKSON, CLARENCE MARTIN, Growth, 297
- JANSSEN, GEORGE, Physical measurements of winter wheat, 477
- JOHNSTON, EARL S., Influence of boron on tomato, 31
- K
- KNIGHT, HUGH, Petroleum oils as insecticides, 299
- KUNG, FAN CHI, Obit., 548
- L
- Laboratory, research, University of Minnesota, 162
- LARSON, CARL, Nitrate nitrogen determination in wheat plants, 288
- Laurocerasus laurocerasus*, translocation of oil, 317
- Leaf sap, expression, critical comments, 103
tissue, moisture fluctuations, 529
- Leaves, mineral content, effect of fertilizing, 269
wheat, water content at flowering, 393
- LEE, ATHERTON, Toxic substance produced by eye-spot fungus in sugar cane, 193
- Lemon, chemical relation of stock and scion, 113
rough, colorimetric identification, 265
- Length of day, and top-root relations, 71, 73
- Lettuce, nitrate determination in green tissue, 519
top-root size relations, 66
- Life membership, award, 161
committee, 399
- Light, ranges of wave length and stomatal opening, 323
- Lignin-suberin, 40
- Limiting factors, in use of petroleum oils as insecticides, 299

- LINCOLN, F. B., Extraction of nitrogen materials from pear tissues, 233
 LLOYD, F. E., Water-tight door of *Utricularia* trap, 87
 LOOMIS, W. E., SCHLOESING's experiments on relation of transpiration to translocation of minerals, 158

M

- MACDOUGAL, D. T., Hydrostatic-pneumatic system of trees, 297
 Maltreatments of pear tissue, 238
 Manganese content, velvet bean seed coat, 512
 Manuscripts, 548
 MAXIMOW, N. A., Physiological basis of drought resistance, 402, 549
 Measurements, physical, of wheat plant, 477
 Mechanism, of cell wall formation, 329 of *Utricularia* trap, 87
 Meeting, fall, Division of colloid chemistry, American Chemical Society, 400
 fifth annual, 161
 Des Moines, 547
 Members, foreign, 547
 Membership, life, committee, 399
 Method, for determining nitrate nitrogen, 288, 519
 TSWETT adsorption, and pure pigments, 337
 Methods, analytical, 37, 288, 374, 412, 466, 519
 MEYER, BERNARD S., Critical comments on methods of expressing sap, 103
 Microchemistry of phloridzin, 357
 MILLER, EMERSON R., Insoluble tyrosinase of velvet bean, 507
 Mineral accumulations, X-ray photography, 145
 content, effect of fertilizing on, 269
 translocation and transpiration, 158
 Minnesota, research laboratory, 162
 Moisture, fluctuations in extracted sap and leaf tissues, 529
 MULAY, A. S., Extraction of nitrogenous materials from pear tissues, 233
 MURNEEK, A. E., Hemicellulose, storage carbohydrate in woody plants, 251

N

- Necrosis, 5
 Nitrate determination, in green tissues, 288, 519
 Nitrites, determination, 197
 Nitrogen content, velvet bean seed coat, 512
 distribution in expressed sap of pear, 249
 effect on chloroplast pigments, mineral content and production, 269
 fixation, atmospheric, ERNST, 403
 nitrate, determination in wheat plant, 288
 Nitrogenous materials, extraction from pear tissues, 233
 Non-protein nitrogen, pear tissues, 242
 Numerical data of biology, TERROINE and COLIN, 163

O

- Oats, root and shoot under long and short day, 448
 Oils, saturated petroleum, as insecticides, 299
 OLIVER, R., Storage of truck crops, girasole, 123
 Orange, chemical relation of stock and scion, 113
 colorimetric determination, 265
 Orcinol, color development, 515
 Osmotic pressure, of winter wheat, 482
 Outlines of biochemistry, GORTNER, 549
 OVERTON, J. B., Hydrostatic-pneumatic system of trees, 297
 Oxidase activity, effect of pH and hydrogen peroxide, 363
 fruit, inactivation temperature, 537

P

- Pacific science congress, fourth, 294
 Pear, 233, 405, 459
 composition of dormant shoots, 405
 seasonal changes in tracheal sap, 459
 tissue, extraction of nitrogenous material, 233
 Penetration of petroleum oils into leaves, 310, 313
 Peroxidase, pH value and inactivation temperatures, 540
 Petroleum oils as insecticides, 299

- PFEFFER, WILHELM**, biographical sketch, 285
 portrait, opposite 285
 portrait obtainable, 400, 547
- Phloridzin**, microchemical demonstration, 357
- Phloroglucinol**, color development, 515
- Phosphates**, absorption, 213
- Phosphorus**, effect on chloroplast pigments, mineral content, and production, 269
- Photography**, X-ray, of mineral accumulations, 145
- Photosynthesis**, and petroleum oil insecticides, 301
- Physical components of plant transpiration**, SEYBOLD, 550
 measurements of wheat plants, 477
- Physiological basis of drought resistance**, MAXIMOW, 402, 549
- Physiology of bacteria**, BUCHANAN and FULMER, 295
- Picea engelmanni*, sap expression, 104
- Pigments**, chloroplast, 165, 269, 337
 and TSWETT adsorption method, 337
- Pinus rigida*, sap expression, 105
- Pisum sativum*, calcium deficiency, 493
- Plane tree**, chlorophyll, 138
- PLANT PHYSIOLOGY**, increase in price of back volumes, 293
- Plants**, woody, hemicellulose storage, 251
- Plant solutions**, moisture fluctuations, 529
- Plaster molds**, growth of roots in, 15
- Portraits**, PFEFFER, opposite 285
 obtainable, 400, 547
- SACHS**, opposite 155
 obtainable, 294, 400, 547
- TIMIRIAZEFF**, obtainable, 294, 400, 547
- VAN HELMONT**, opposite 543
 obtainable, 547
- Potassium**, effect on chloroplast pigments, mineral content, and production, 269
 on top and root size, 66
- Potato**, influence of shape on chemical composition, 385
 leaf analyses, chloroplast pigments and fertilizer elements, 275
 sprout, invading, 157
- Preparation of samples**, 37
- Preservation**, influence on nitrogenous materials of pear, 233
- Pressure**, effects on cells of root tip, 13
 lateral, on root growth, 18
- Production**, effect of fertilizing on, 269
- Program committee**, 399
- Properties and actions**, enzymes, WALDSCHMIDT-LEITZ, 296
- Protein nitrogen of pear tissues**, 242
- Protoplasm**, colloid chemistry, HEILBRUNN, 164
- Pruning**, and top-root relation, 79
- Prunus cerasifera*, pigments, 137
demissa, phloridzin, 358
- Purdue section**, activities, 400
- Pyridine** as stain carrier for fats, 307
- Pyrocatechol**, color development, 515
- Pyrogallol**, color development, 516

Q

Quercus agrifolia, and petroleum oils, 314

R

- Radish**, top and root relations, 73
 root and shoot under long and short day, 441
- Ragweed**, great, root and shoot under long and short day, 446
- Ratios**, area-weight of tomato, 351
 length-width, etc., of potato, 388
- Ray filters**, 324
- Reaction value**, and inactivation temperature of fruit oxidase, 537
- Reduction of nitrate**, 526
- REED, ERNEST**, Testing sugar beets for genetic purposes, 367
- Representative**, council A. A. S., 293
- Research laboratory**, University of Minnesota, 162
- Resistance**, drought, physiological basis, MAXIMOW, 402, 549
- Respiration**, and petroleum oil insecticides, 301
 of tomato fruits, 349
- Restitution of roots**, 5
- RICKETT, HAROLD W.**, Botany, 402
- ROBBINS, W. J.**, Botany, 402
 growth, 297
- Root and top**, size relations, 63
 system, development in long and short day, 435
 tips, effect of pressure, 13

- Roots, growth in plaster molds, 15
under lateral pressure, 18
Rumex patientia, stomatal behavior, 323

S

- Saccharum officinarum*, X-ray photography, 145
SACHS, JULIUS VON, biography, 155
portrait, opposite 155
obtainable, 294, 400, 547
Salt series, order of effectiveness in protein extraction, 246
SAMEC, M., Colloid chemistry of starch, 164
SAMUELS, CHARLES D., Petroleum oils as insecticides, 299
Sap, expression from leaves, critical comments, 103
wheat, expressed, 222
Saturated petroleum oils as insecticides, 299
SAYRE, J. D., Opening of stomata in different wave lengths of light, 323
SCHERTZ, F. M., Seasonal variation of chloroplast pigments, 135
Effect of fertilizing on chloroplast pigments, mineral content, and production, 269
Pure pigments and TSWETT adsorption method, 337
SCHLOESING'S experiments on translocation of minerals, 158
SCHOEN, M., Problem of fermentation, translation by HIND, 163
Science congress, fourth Pacific, 294
Scion and stock, chemical relationship, citrus, 113
Seasonal changes in sap of pear and apricot, 459
variation of chlorophyll, 135
Section, Purdue, activities, 400
Seed coat, velvet bean, insoluble tyrosinase, 507
Seeds, forest tree, for research, 162
Seventh colloid symposium, 294
SEYBOLD, A., Physical components of plant transpiration, 550
Shape, influence on chemical composition of potato, 385
SHIVE, J. W., Chloroplast pigments and dry weights of tops of corn, 165
Shoot, development under long and short day, 435
Shoots, dormant pear, composition of, 405
SMITH, GILBERT M., Hydrostatic-pneumatic system of trees, 297
Soil cultures, absorption of phosphate from, 213, 214
Solution cultures, absorption of phosphate from, 213, 219
Specific resistance, pear and apricot sap, 463
SPOEHR, H. A., Life membership award, 161
SPONSLER, O. L., Mechanism of cell wall formation, 329
SPRAGUE, H. B., Chloroplast pigments, and dry weights of tops of corn, 165
Sprout, potato, invading, 157
Starch, 39, 413
Colloid chemistry of, SAMEC, 164
STEPHEN HALES prize award, 162
Stinging nettle, chlorophyll, 138
Stock and scion, citrus, chemical relationship, 113
Stomata, opening, in light of different wave lengths, 323
Storage, changes in alcoholic extracts during, 141
girasole, 123
hemicellulose, in woody plants, 251
STOUT, G. J., Top and root size in herbaceous plants, 63
STRAUSBAUGH, P. D., Invading potato sprout, 157
Structure, roots of *Pisum sativum*, calcium deficiency, 500
stems of *Pisum sativum*, calcium deficiency, 501
Studies in experimental cytology, 1
Substance, toxic, eye-spot fungus, 193
Sucrose, 412
Sugar cane, eye-spot fungus, toxic to, 193
content of beets, testing, 367
Sunflower, root and shoot under long and short day, 443
Sulphur, and root-top relationship, 66
Superphosphate, and root-top relationship, 66, 69

SVEDBERG, THE, Colloid chemistry, 2nd ed., 297

Symposium, seventh colloid, 294

System, hydrostatic-pneumatic, of trees, MACDOUGAL, OVERTON, and SMITH, 297

root, development under long and short day, 435

T

Tables, international critical, 294

TAYLOR, J. W., Water content of wheat leaves at flowering, 393

TEAKLE, L. J. H., Absorption of phosphate from soil and solution cultures, 213

Temperature, cold, preservation of pear tissue, 237

effect on flowers, 281

inactivation of fruit oxidase, 537

influence on dormant pear shoots, 405

TERROINE, E. F., Numerical data of biology, 163

Testing sugar content of beets, 367

Thawing rate and injury of wheat, 479

Thermonasty, crocus and tulip, 281

Threshold of *Utricularia* trap, 92

THOR, CLIFFORD J., Storage of truck crops, girasole, 123

Tissues, pear, extraction of nitrogenous materials, 233

Titration curves, wheat sap, 224

Tomato, determination of nitrate in green tissue, 519

influence of boron on composition and growth, 31

respiration of fruits, 349

top-root relation, 77

Top and root size relation, 63, 77, 435

Toxic substance, eye-spot fungus, 193

Tracheal sap, pear and apricot, seasonal changes, 459

Translocation of minerals, and transpiration, SCHLOESING's experiments, 158 of petroleum oils, 306, 313

Transmission of ray filters, 326

Transpiration, 300

and mineral translocation, SCHLOESING's experiments, 158

plant, physical components, SEYBOLD, 550

Trap of *Utricularia*, mechanism, 87

TRAUB, HAMILTON P., Storage of truck crops, girasole, 123

Tree seeds, forest, 162

Trees, hydrostatic-pneumatic system, MACDOUGAL, OVERTON, and SMITH, 297

TRIER-WINTERSTEIN, The alkaloids, 164

Truck crops, storage, girasole, 123

TSWETT adsorption method, and pure pigments, 337

Tubers, potato, shape and chemical composition, 385

Tyrosinase, insoluble, in velvet bean, 507

U

Uranium acetate, phloridzin reagent, 357

Uronic acids, in hemicellulose, 374

Utricularia trap, mechanism, 87

V

VAN HELMONT, JOANNES BAPTISTA, biographical sketch, 543

portrait, opposite 543

obtainable, 547

Velvet bean, insoluble tyrosinase, 507

Volatility of petroleum oils, 318

W

WALDSCHMIDT-LEITZ, ERNST, Enzyme actions and properties, 296

Wall formation, mechanism of, 329

WALTON, R. P., Translation of WALDSCHMIDT-LEITZ, Enzyme actions and properties, 296

Water content of wheat leaves at flowering, 393

Wave-length range, and opening of stomata, 323

WEAVER, J. E., Root and shoot relation under long and short day, 435

WEBSTER, JAMES E., Effects of storage on plant extracts, amino acid changes, 141

Wheat, absorption of phosphate from soil and solution cultures, 213 determination of nitrate nitrogen in, 288

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before

Return Date	Return Date